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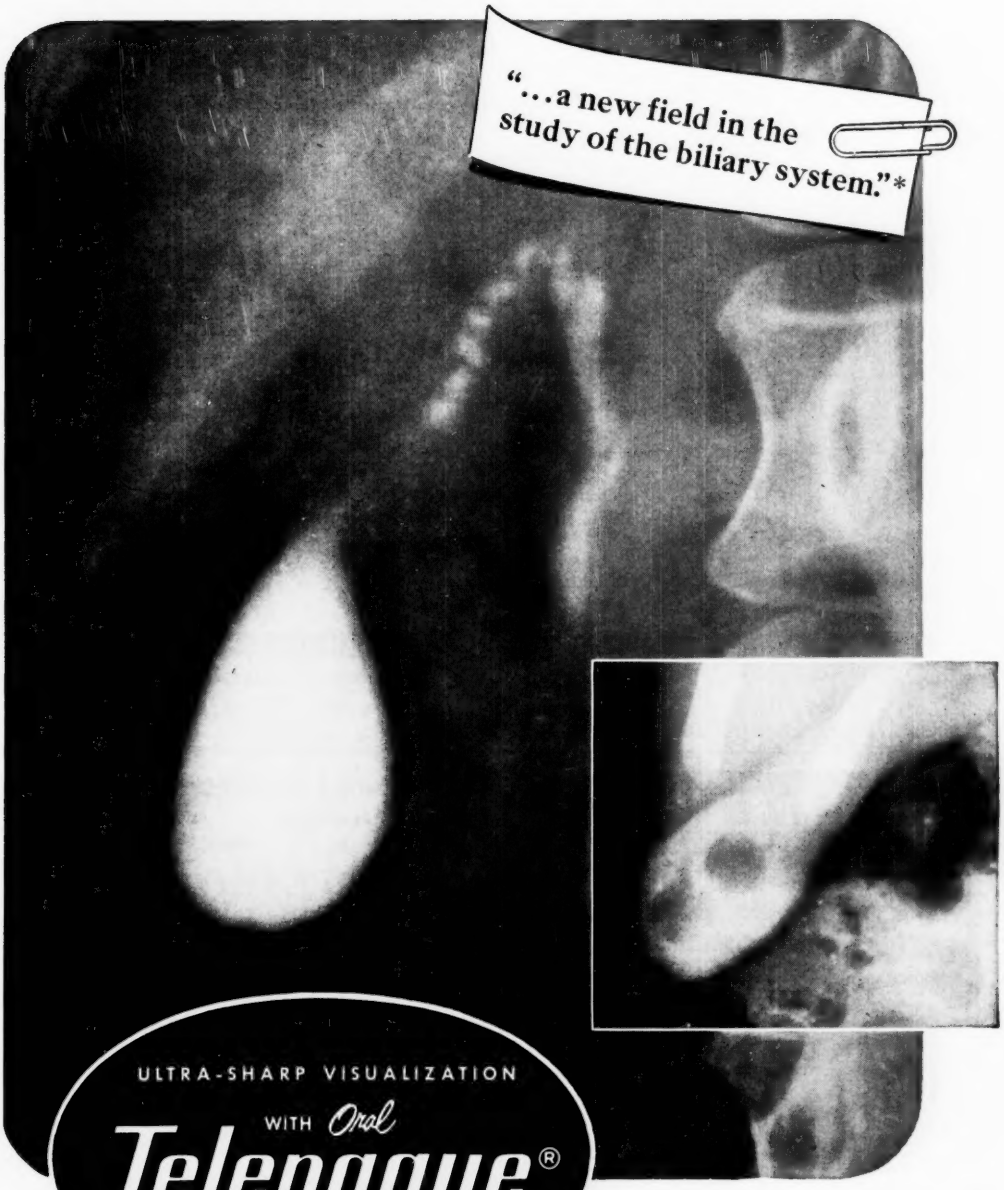

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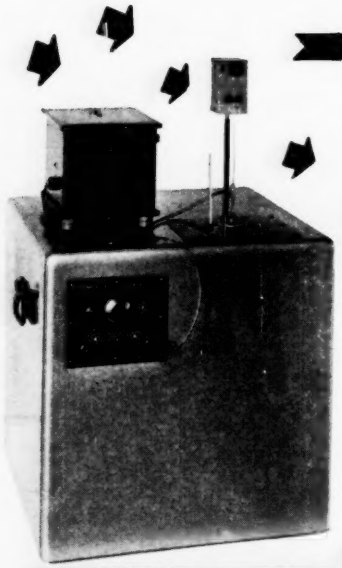

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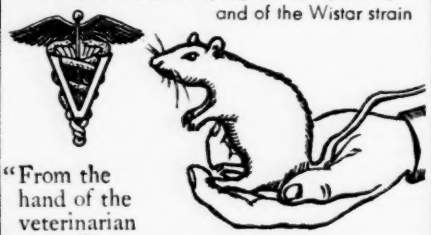

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1. Gross, F., and Tschopp, E.: Experientia *8*:75, 1952. **2.** Thorn, G. W., and Jenkins, D.: In press. **3.** Thorn, G. W.; Jenkins, D.; Arons, W. L., and Frawley, T. F.: Schweiz. med. Wchnschr. *82*:697, 1952. **4.** Gaunt, R.; Leathem, J.; Howell, C., and Antonchak, N.: Endocrinology *50*:521, 1952. **5.** Sorkin, S. Z., and Soffer, L. J.: Am. Fed. Clin. Research, May 4, 1952.

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THE REACTION OF THE CAPILLARY BED OF THE NAILFOLD TO THE CONTINUOUS INTRAVENOUS INFUSION OF LEVO-NOR-EPINEPHRINE IN PATIENTS WITH NORMAL BLOOD PRESSURE AND WITH ESSENTIAL HYPERTENSION

By SHELDON E. GREISMAN

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(Submitted for publication June 12, 1953; accepted March 3, 1954)

The intravenous infusion of l-nor-epinephrine in persons with normal cardiovascular systems produces a hemodynamic state which in many respects resembles that found in patients with essential hypertension. In the systemic circulation there is in each of the two conditions an elevation of the systolic, diastolic and mean arterial pressures, an unchanged or moderately decreased cardiac output, an increase in systemic peripheral resistance and usually an absence of subjective symptoms (1). This similarity in the two hemodynamic states has led to the suggestion that l-nor-epinephrine may be a humoral agent responsible for essential hypertension. If this were true, then the hypertensive state produced by l-nor-epinephrine should produce changes in all portions of the circulatory system similar to those found in essential hypertension. The dynamics of the pulmonary circulation have been shown to be different in the two states; the intravenous infusion of l-nor-epinephrine raises mean pulmonary arterial pressure whereas this pressure is normal in patients with uncomplicated essential hypertension (1).

The present study was undertaken to determine whether the circulation in specific portions of the systemic vascular system, in this instance the peripheral cutaneous capillaries, is the same in subjects with l-nor-epinephrine induced hypertension as in patients with essential hypertension. Accordingly, the capillary circulation of the nailfold during the sustained hypertensive state induced in control subjects by continuous intravenous infusion of l-nor-epinephrine was compared with the nailfold capillary circulation of patients with essential hypertension. The observations indicate that for similar levels of elevated arterial pressure the capillary circulation of the nailfold is more reactive in the l-nor-epinephrine induced hypertension than in essential hypertension. A previous study indicated that the nailfold capillary circulation of subjects with essential hypertension was more reactive to single rapid injections of epinephrine than the similar capillary circulation of control subjects (2). The present study extends this observation to indicate that such hyper-reactivity persists over the several hours of a continuous infusion of l-nor-epinephrine.

METHOD

Two groups of hospitalized patients were selected; one with normal cardiovascular systems, the other with essential hypertension. The hypertensive patients had known essential hypertension for many years or had been followed on the wards for several weeks and had generally maintained diastolic blood pressure levels of 90 mm. Hg or higher. Patients receiving medication which could produce cardiovascular changes were excluded. An attempt was made to select patients of relatively similar ages in the two groups. During the observations the subjects were recumbent with a light blanket over the trunk and lower extremities. Patients with cool, pale, or cyanotic fingers were not studied. One arm was abducted in the horizontal plane approximately thirty degrees from the side, with the terminal phalanx of the fourth finger at about the level of the sternum. The finger was steadied by a clamp applied along the lateral margins of the phalanx. Cedar oil was applied to the base of the nail bed and the terminal capillary loops were then readily visualized microscopically at 96 × when illuminated by a slit lamp. A blood pressure cuff was placed about the opposite arm and an infusion of isotonic salt solution was begun in an antecubital vein in such a manner that there was no interference with auscultatory blood pressure determinations. Following these procedures twenty minutes were allowed to elapse, or until the blood pressure had stabilized. If at the end of this period the fingers had become cool, pale, or cyanotic, or if the blood flow through the capillary loops had slowed, as visualized microscopically, another twenty minutes were allowed to elapse. If the fingers did not become warm and the capillary blood flow did not become rapid, no observations were made. After the

TABLE I

Alteration of blood pressure, pulse rate, and vasomotion of the capillary bed of the fingernail during l-nor-epinephrine infusion in patients with normal blood pressure

Patient no.	Age	Sex	Diagnosis	Rate of norepinephrine infusion (*gamma Kg./minute*)	Arterial blood pressure (*mm. Hg*)	Pulse rate (*per minute*)	Intensity of vasomotion of the capillary bed of nailfold*	Duration of infusion (*minutes*)
1	56	F	Pulmonary fibrosis and emphysema	—	110/54	84	0	—
				.05	120/56	84	0	5
				.10	118/60	80	×	5
				.17	146/72	74	2×	10
				.23	170/80	72	3×	10
				.30	180/80	72	Initial ischemia	10
				.32	180/80	60	Ischemia	40
2	50	M	Recovered barbiturate intoxication	—	106/58	72	1×	—
				.03	106/58	72	1×	5
				.06	106/58	72	1×	5
				.07	108/64	72	2×	10
				.09	112/68	70	2×	5
				.13	120/76	68	3×	10
				.17	130/80	68	Initial ischemia	15
				.26	140/84	64	Ischemia	15
				.29	150/90	58	Ischemia	30
3	35	M	Normal male	—	110/60	74	×	—
				.12	120/74	64	3×	10
				.15	120/74	64	Initial ischemia	20
4	56	M	Chronic alcoholism	—	114/60	72	×	—
				.03	114/62	72	1×	5
				.21	130/70	60	3×	10
				.30	150/80	56	Initial ischemia	20
				.52	160/80	48	Ischemia	25
5	27	M	Pharyngitis (convalescent)	—	126/60	76	1×	—
				.02	120/60	76	1×	5
				.06	126/60	76	1×	5
				.10	124/64	76	2×	10
				.12	126/70	76	2×	10
				.20	130/70	74	Initial ischemia	10
				.25	136/74	72	Ischemia	10
				.31	140/70	70	Ischemia	20
6	47	F	Barbiturate addiction	—	100/70	76	×	—
				.08	108/74	76	×	10
				.20	120/80	72	2×	10
				.32	140/88	70	Initial ischemia	30
7 a)	38	F	Chronic alcoholism	—	110/60	84	1×	—
				.04	110/60	84	2×	15
				.11	130/76	84	3×	15
				.22	140/88	84	Initial ischemia	75
b)			Same patient 3 days later	—	110/70	84	1×	—
				.22	140/88	72	Initial ischemia	180
8	22	F	Normal female	—	120/70	72	0	—
				.05	120/70	72	1×	5
				.09	126/80	72	2×	10
				.13	130/90	66	Initial ischemia	20
9 a)	74	M	Esophageal carcinoma	—	154/70	80	×	—
				.04	150/70	80	×	5
				.06	150/72	80	×	5
				.08	180/84	76	2×	10
				.12	200/88	74	3×	10
				.28	220/100	70	Initial ischemia	30

* Average number of constrictions of the arterial segments of the capillary loops per minute. 0 = 0–2, × = 3–8, 1× = 9–14, 2× = 15–25, 3× = 25–30.

TABLE I—*Continued*

Patient no.	Age	Sex	Diagnosis	Rate of norepinephrine infusion (*gamma/Kg./minute*)	Arterial blood pressure (*mm. Hg*)	Pulse rate (*per minute*)	Intensity of vasomotion of the capillary bed of nailfold*	Duration of infusion (*minutes*)
9 b)			Same patient	—	130/70	72	×	—
			5 days later	.03	130/70	72	×	5
				.06	140/76	72	1×	5
				.10	170/80	66	2×	5
				.20	180/90	64	Initial ischemia	25
10	33	F	Recovered	—	116/76	92	1×	—
			barbiturate	.02	116/76	92	1×	5
			intoxication	.07	120/78	92	2×	5
				.10	120/80	92	3×	5
				.13	130/86	90	Initial ischemia	5
				.17	146/86	88	Ischemia	5
				.20	146/86	84	Ischemia	120
11	67	M	Resolved	—	140/76	76	1×	—
			broncho	.06	150/80	76	2×	5
			pneumonia	.12	160/80	74	3×	5
				.24	190/88	68	Initial ischemia	10
				.37	220/90	60	Ischemia	15
				.48	240/100	56	Ischemia	20
12	36	F	Chronic	—	130/80	88	2×	—
			alcoholism	.09	130/84	84	3×	5
				.17	150/90	72	Initial ischemia	10
				.22	150/94	68	Ischemia	10
				.26	160/100	60	Ischemia	15
				.34	170/110	56	Ischemia	25
13	77	M	Chronic gout	—	140/80	84	×	—
				.07	150/86	84	1×	5
				.12	180/86	80	2×	5
				.17	190/86	76	3×	10
				.27	220/96	72	Initial ischemia	60
14	53	F	Recovered	—	142/80	72	×	—
			barbiturate	.19	190/94	68	3×	5
			intoxication	.33	190/98	60	Initial ischemia	15
				.40	220/112	56	Ischemia	25
15	63	M	Pulmonary	—	130/90	72	1×	—
			fibrosis and	.16	186/108	72	2×	5
			emphysema	.20	212/116	70	3×	10
				.40	240/120	70	Initial ischemia	30

control period a solution of l-nor-epinephrine,[1] 4 gamma per cubic centimeter in isotonic saline solution, was then substituted for the original saline solution, without disturbing the patient, by changing the infusion bottle. The rate of infusion of the l-nor-epinephrine was increased progressively and with each increment the arterial blood pressure, the pulse rate, and the reaction of the terminal capillary loops at the base of the fingernail were recorded. The rates and duration of infusion of l-nor-epinephrine are indicated in Tables I and II.

RESULTS

Reactivity of capillary bed

The terminal capillary loops of the nailfold of the finger were usually patent with a rapid blood flow in both normotensive and hypertensive subjects. Intermittent momentary interruptions of blood flow occurred in the arterial segments of the capillary loops. The cause of these interruptions in blood flow could not be determined directly since the vascular bed proximal to the terminal capillary loops could not be visualized clearly. However, when the capillary beds of human bulbar conjunctivae and of the mesenteries of mice were observed, similar, though less frequent interruptions in capillary blood flow were seen. These gaps in blood flow were observed to be produced by intermittent constriction of the metarterioles and precapillary segments of these vascular beds and epinephrine was seen to increase the frequency of constriction of these metarterioles and precapil-

[1] Bitartrate salt (synthetic: supplied by Winthrop-Stearns, Inc.).

TABLE II

Alteration of blood pressure, pulse rate, and vasomotion of the capillary bed of the fingernail during l-nor-epinephrine infusion in patients with essential hypertension

Patient no.	Age	Sex	Diagnosis	Rate of norepinephrine infusion (*gamma/Kg./minute*)	Arterial blood pressure (*mm. Hg*)	Pulse rate (*per minute*)	Intensity of vasomotion of the capillary bed of nailfold*	Duration of infusion (*minutes*)
1	46	F	Hypertensive	—	146/86	72	2×	—
			vascular	.04	160/90	72	2×	10
			disease	.07	172/98	72	3×	10
			(known 10 years)	.13	184/104	72	Initial ischemia	10
				.16	190/110	70	Ischemia	10
2	68	F	1) Hypertensive	—	154/88	88	2×	—
			vascular	.06	160/88	88	3×	10
			disease	.11	166/94	88	Initial ischemia	15
			(unknown	.16	170/96	88	Ischemia	15
			duration)	.26	188/98	88	Ischemia	15
			2) Old cerebro-vascular accident					
3	54	F	1) Hypertensive	—	170/86	60	1×	—
			vascular	.04	170/86	60	3×	15
			disease	.07	190/86	51	Initial ischemia	15
			(known 11 years)	.10	212/96	48	Ischemia	15
			2) Hypertensive cardiovascular disease II B	.13	230/96	45	Ischemia	15
4	52	F	1) Hypertensive	—	150/90	92	2×	—
			vascular	.05	150/90	88	3×	15
			disease	.08	170/98	88	Initial ischemia	15
			(known 9 years)	.17	200/108	86	Ischemia	10
			2) Old myocardial infarction					
5	60	M	1) Hypertensive	—	190/90	72	3×	—
			vascular	.05	194/90	72	Initial ischemia	10
			disease	.11	210/90	60	Ischemia	10
			(unknown	.21	240/110	57	Ischemia	10
			duration)					
			2) Cerebral arteriosclerosis					
6	73	F	1) Hypertensive	—	190/94	100	2×	—
			vascular	.05	198/100	96	Initial ischemia	15
			disease	.09	208/100	100	Ischemia	10
			(known 15 years)	.18	230/120	96	Ischemia	10
			2) Old cerebro-vascular accident	.25	238/120	84	Ischemia	10
7	67	F	Hypertensive	—	220/94	84	2×	—
			vascular	.06	240/90	80	2×	10
			disease	.09	248/94	84	Initial ischemia	15
			(known 10 years)	.16	284/94	84	Ischemia	10
8	71	F	1) Hypertensive	—	220/100	88	3×	—
			vascular	.07	220/106	88	Initial ischemia	10
			disease	.22	220/110	80	Ischemia	10
			(unknown	.31	240/120	80	Ischemia	30
			duration)	.71	240/120	76	Ischemia	5
			2) Bronchogenic carcinoma					
9	66	F	1) Hypertensive	—	212/106	72	2×	—
			vascular	.04	220/108	72	3×	5
			disease	.06	232/100	72	Initial ischemia	5
			(unknown	.09	244/104	72	Ischemia	10
			duration)	.11	258/110	72	Ischemia	55
			2) Old cerebro-vascular accident					

* Average number of constrictions of the arterial segments of the capillary loops per minute. 0 = 0–2, × = 3–8, 1× = 9–14, 2× = 15–25, 3× = 25–30.

TABLE II—*Continued*

Patient no.	Age	Sex	Diagnosis	Rate of norepinephrine infusion (*gamma/Kg./minute*)	Arterial blood pressure (*mm. Hg*)	Pulse rate (*per minute*)	Intensity of vasomotion of the capillary bed of nailfold*	Duration of infusion (*minutes*)
10 a)	52	M	1) Hypertensive	—	210/110	88	×	—
			vascular	.06	210/110	88	3×	10
			disease	.08	210/116	88	Initial ischemia	10
			(known 6 years)	.12	220/116	80	Ischemia	10
			2) Old cerebral	.19	220/118	80	Ischemia	10
			thrombosis	.24	234/120	80	Ischemia	10
b)			Same patient	—	190/102	88	2×	—
			10 days later	.09	200/100	88	Initial ischemia	10
				.15	200/110	84	Ischemia	10
				.25	246/130	76	Ischemia	10
11	60	F	1) Hypertensive	—	210/110	100	3×	—
			vascular	.03	210/110	88	Initial ischemia	15
			disease	.05	226/110	88	Ischemia	15
			(known 5 years)	.09	220/110	88	Ischemia	10
			2) Acute gastritis	.13	220/110	88	Ischemia	10
			(subsided)	.17	220/116	88	Ischemia	10
				.28	236/116	88	Ischemia	10
12	52	F	Hypertensive	—	210/120	80	2×	—
			vascular	.03	226/120	72	3×	10
			disease	.07	240/118	72	3×	10
			(unknown	.09	250/130	72	Initial ischemia	10
			duration)	.11	250/134	72	Ischemia	10
				.15	260/130	72	Ischemia	15
13	66	F	1) Hypertensive	—	240/130	84	3×	—
			vascular	.05	260/134	84	Initial ischemia	10
			disease	.10	270/136	84	Ischemia	15
			(known 3 years)					
			2) Osteoarthritis					
14	57	F	1) Hypertensive	—	220/132	100	3×	—
			vascular	.08	236/138	100	Initial ischemia	10
			disease	.14	238/140	100	Ischemia	15
			(known 15 years)	.23	240/140	100	Ischemia	15
			2) Cerebrovascular accident					
15	38	F	Hypertensive	—	240/140	84	3×	—
			vascular	.08	240/140	84	Initial ischemia	15
			disease	.16	250/140	84	Ischemia	15
			(known 5 years)	.32	250/144	80	Ischemia	20

laries. Epinephrine was also observed to increase the frequency of interruptions of blood flow through the terminal capillary loops of the nailfold. For these reasons the interruptions of blood flow through the capillary loops of the nailfold were interpreted as the result of irregular, periodic vasoconstriction of the minute vascular bed. The relative contribution of the arterioles, metarterioles, and arterial segments of the capillary loops in the production of these gaps in blood flow could not be determined. (The proximal portions of the arterial segments of the capillary loops probably were capable of active vasoconstriction since the interruptions in blood flow occurred independently in adjacent capillary loops.) The process of intermittent vasoconstriction of the minute vascular bed has been termed "vasomotion" (3–5).

In patients with normal blood pressure, there were usually 3 to 14 interruptions of blood flow through the arterial segment of the capillary loops per minute (denoted in Table I under Intensity of Vasomotion by × and 1 ×). Each cessation of blood flow rarely exceeded two seconds in duration. In patients with essential hypertension there was usually a definite increase in the *frequency* of interruptions of blood flow through the capillary loops, often averaging 15 to 30 cessations of flow per minute (denoted in Table II under Intensity of Vasomotion by *2* × and 3 ×). The *duration* of each interruption in flow was as transient as that in persons with normal blood pressure.

L-nor-epinephrine infusion, normotensive subjects (Table I)

When l-nor-epinephrine was infused intravenously in patients with normal blood pressure, both the systolic and diastolic pressures increased as the rate of infusion increased. Concomitantly, vasomotion increased progressively as indicated by the increasing frequency of cessations of capillary blood flow. The duration of each interruption of blood flow increased but slightly, so that the resulting ischemia of the arterial segment was not maintained for intervals longer than two seconds. A stage was finally attained during which the capillary bed appeared similar to that observed in patients with essential hypertension. This stage was reached when the diastolic pressure had been elevated an average of 11 mm. Hg (range from 4 to 26 mm. Hg) to levels of 70 to 90 mm. Hg (Table I).

When the rate of l-nor-epinephrine infusion was increased further, not only did the frequency of interruptions of blood flow through the arterial segments of the capillary loops increase, but the duration of the cessations of flow became progressively longer until an "ischemic state" developed which no longer resembled the capillary blood flow seen in patients with essential hypertension. The entire capillary loop (not the arterial segment alone) now disappeared from view for periods of two seconds to several hours. At times complete cessation of blood flow through the capillary loop occurred so that stagnated clumps of red blood cells marked the outline of the original blood vessel. The ischemic state persisted virtually unchanged as long as the infusion continued.

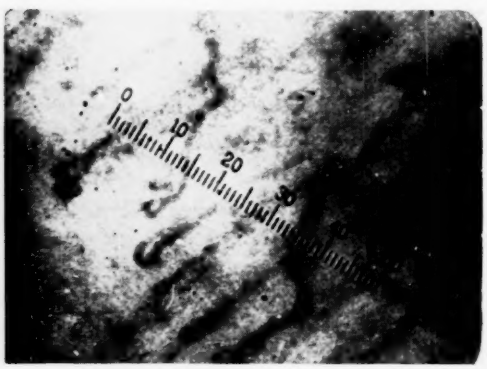

FIG. 1. CAPILLARY BED OF THE NAILFOLD OF A PATIENT WITH NORMAL BLOOD PRESSURE (68×) B.P. = 126/60 P.R. = 76

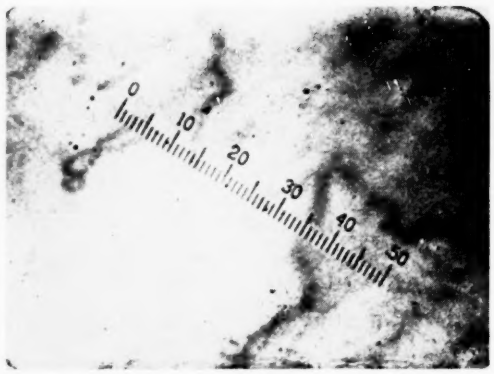

FIG. 2. INITIAL ISCHEMIC REACTION OF THE CAPILLARY BED DURING L-NOR-EPINEPHRINE INFUSION (68×) 0.20 GAMMA/KG./MINUTE B.P. = 130/70 P.R. = 74 (40 MINUTES AFTER START OF INFUSION)

The ischemic reaction did not occur simultaneously in all the capillary loops. Of an average of ten loops usually visualized in any one microscopic field, ischemia occurred initially and persisted in two or three capillary loops, generally those adjacent to each other and of the narrowest caliber. As the rate of infusion was increased, increasing numbers of capillary vessels reacted similarly. The point at which ischemia occurred in two or three of the capillary loops in one microscopic field was selected as the point of "initial ischemic reaction." In persons with normal cardiovascular systems, initial ischemia of the capillary bed of the nailfold developed when the rate of l-nor-epinephrine infusion raised the diastolic pressure an average of 17 mm. Hg (range from 10 to 30 mm. Hg) to levels of 80 to 100 mm. Hg. The ischemic reaction persisted virtually unchanged for the duration of the infusion, twenty minutes to three hours. Capillary ischemia was not usually seen in patients with essential hypertension, even at diastolic pressure levels of 140 mm. Hg.

In fifteen patients with normal blood pressures the mean rate of infusion of l-nor-epinephrine required to produce an initial ischemic reaction was 0.23 gamma per Kg. per minute (standard deviation of 0.07). Figure 1 is a photomicrograph (68×) of the capillary bed of the nailfold of a patient with normal blood pressure. Figure 2

indicates the initial ischemic reaction of the capillary bed during the infusion of l-nor-epinephrine.

L-nor-epinephrine infusion, hypertensive patients (Table II)

When l-nor-epinephrine was infused in patients with essential hypertension, both the systolic and diastolic pressures increased as the rate of infusion was increased. The rise in systolic pressure with low rates of infusion was significantly greater than the rise which occurred in patients with normal blood pressures (Table III). As the rate of infusion was increased, however, the rise of systolic pressure became progressively less marked, as compared to the continuing rise of systolic pressure in patients with normal blood pressures. There was no significant difference in the rate of elevation of the diastolic or mean arterial pressures in patients with essential hypertension and with normal blood pressures (Table III).

As l-nor-epinephrine was infused in patients with hypertensive vascular disease, vasomotion of the minute vessel bed, initially greater than normal, increased so rapidly that an ischemic reaction occurred with low rates of infusion (Table II). Again the "disappearance" of capillary loops and the stagnation of clumps of red blood cells in adjacent vessels did not occur simultaneously. Usually two or three loops in any one microscopic field were involved initially. As the rate of infusion was increased, progressively more capillary loops became ischemic. In patients with essential hypertension, the initial ischemic reaction of the capillary bed occurred when the rate of the l-nor-epinephrine infusion had raised the diastolic pressure an average of 4 mm. Hg (range from 0 to 18 mm. Hg) from the initial level. In fifteen patients with essential hypertension, the mean rate of infusion of l-nor-epinephrine required to produce an initial ischemic reaction of the capillary bed

TABLE III

Resting levels and increases of systemic arterial pressures during l-nor-epinephrine infusion in patients with normal blood pressure and with essential hypertension

	Normal blood pressure				Essential hypertension			
	No. cases	Mean	Standard deviation	Range	No. cases	Mean	Standard deviation	Range
I. Resting levels								
Systolic pressure, *mm. Hg*	15	122	13	100–142	15	200	31	146–240
Diastolic pressure, *mm. Hg*	15	70	10	54–90	15	105	16	86–140
Mean pressure, *mm. Hg*	15	90	10	76–106	15	141	22	110–180
II. Increase of pressures with increasing doses of l-nor-epinephrine								
0.05–0.07, *gamma/Kg./minute*								
Systolic pressure, *mm. Hg*	15	6.1	4.2	0–10	15	14.0	8.4	0–30
Diastolic pressure, *mm. Hg*	15	3.5	2.2	0–7	15	2.5	3.5	0–12
Mean pressure, *mm. Hg*	15	5.0	3.0	0–8	15	6.7	4.4	0–18
(t for systolic blood pressure changes in patients with normal blood pressures as compared with those of essential hypertension = 3.2)								
0.10–0.13, *gamma/Kg./minute*								
Systolic pressure, *mm. Hg*	15	16.0	12.0	0–40	15	24.0	15.4	5–60
Diastolic pressure, *mm. Hg*	15	8.8	5.0	4–20	15	5.5	4.5	0–18
Mean pressure, *mm. Hg*	15	12.0	5.8	4–22	15	13.8	8.7	2–30
(t for systolic blood pressure changes in patients with normal blood pressures as compared with those of essential hypertension = 1.5)								
0.16–0.20, *gamma/Kg./minute*								
Systolic pressure, *mm. Hg*	15	30.0	15.4	4–56	13	35.0	20.6	10–70
Diastolic pressure, *mm. Hg*	15	14.7	5.5	6–25	13	11.4	8.0	0–26
Mean pressure, *mm. Hg*	15	21.5	7.6	8–36	13	20.6	11.0	2–34
(t for systolic blood pressure changes in patients with normal blood pressures as compared with those of essential hypertension = 0.7)								

was 0.07 gamma per Kg. per minute (standard deviation of 0.025).

DISCUSSION

The total systemic peripheral blood flow in patients with essential hypertension is not significantly different from the blood flow in persons with normal blood pressure (6, 7). In one aspect of the peripheral blood flow, the capillary circulation of the nailfold of the finger, a specific difference in the hypertensive patient is noted. Vasomotion, the irregular momentary cessations of capillary blood flow which probably reflects periodic, irregular vasoconstriction of the contractile elements of the minute vascular bed, is usually accelerated in patients with essential hypertension. The infusion of l-nor-epinephrine appeared to intensify vasomotion. When l-nor-epinephrine was infused intravenously in persons with normal blood pressures, the capillary circulation of the nailfold began to resemble that observed in patients with essential hypertension. There was, however, a significant difference. Vasomotion of the capillary bed to a degree resembling that seen in patients with essential hypertension was produced by l-nor-epinephrine when the diastolic blood pressure levels were still within the normal range. Attempts to increase the diastolic blood pressure to levels found in patients with essential hypertension produced a progressive and persistent ischemia of the capillary bed, an appearance not usually seen in patients with essential hypertension. Apparently the diastolic hypertensive state associated with the infusion of l-nor-epinephrine may have central systemic hemodynamics similar to those observed in the diastolic hypertensive state associated with essential hypertension but not a similar nailfold capillary circulation.

The capillary bed of the nailfold of patients with essential hypertension appeared significantly more reactive to l-nor-epinephrine than the same capillary bed in patients with normal blood pressure, as judged by the rates of infusion required to produce initial ischemia of the capillaries ($t = 8.4$). This could not be attributed solely to the increased intensity of vasomotion initially present in persons with essential hypertension whereby only a slight additional vasoconstriction induces an ischemic reaction. As indicated in Table II, vasomotion in patients number 3 and 10a was not more intense initially than in the average patient with a normal blood pressure. The quantity of l-nor-epinephrine required to induce ischemia, however, fell in the same range as that required by hypertensive patients with initially increased vasomotion. This hyper-reactivity of the capillary bed of the nailfold of patients with essential hypertension to sustained intravenous infusions of l-nor-epinephrine conforms with the previously reported hyper-reactivity to single injections (2).

The reaction of the circulation in the capillary bed of the nailfold proved to be a more sensitive index for detecting small quantities of intravenously injected l-nor-epinephrine than was the reaction of the arterial blood pressure, as determined by the auscultatory method. Striking alterations in the capillary blood flow during the lower rates of l-nor-epinephrine infusion were often accompanied by absent or minor alterations in the blood pressure (Table I and II). This was especially noted in patients with essential hypertension.

The elevations in blood pressure and the effects induced in the capillary bed of the nailfold by the infusion of l-nor-epinephrine represented acute changes, for periods not exceeding three hours. During this period, the capillary bed exhibited few signs of adaptation. Even during marked ischemia there was no tendency to return toward the normal state as long as the infusion of l-nor-epinephrine continued at a steady rate. It is possible, however, that l-nor-epinephrine, acting over an extended period, could produce a chronic hypertensive state without inducing a chronic ischemic reaction in the capillary bed of the nailfold. A study of the capillary bed in patients with persistent hypertension due to pheochromocytoma would, therefore, be of interest.

It would be tempting to conclude that the reactions of the capillary circulation of the nailfold here reported indicate the reactivity of the capillary circulation as a whole. In support of such a conclusion one might cite observations which indicate that the reactions of the capillaries of two other areas, the mesoappendix of the rat (8) and the bulbar conjunctivae of man (9), are similar to those here noted for the nailfold. However, a conclusion that the systemic capillary circulation as a whole is more reactive in l-nor-epinephrine induced hypertension than in essential hypertension

is specifically avoided. The variations in capillary reactivity in different vascular areas are too great to permit generalizations from observations on restricted capillary fields. Accordingly, the conclusion of this study remains that for comparable levels of diastolic blood pressure the capillary circulation of the nailfold of the finger is more reactive during l-nor-epinephrine induced hypertension than in essential hypertension. Furthermore the present observations were made in patients of the middle and older age groups with associated disease states. The results, therefore, are not necessarily applicable to young healthy adults or to young adults with uncomplicated essential hypertension.

CONCLUSIONS

1. The circulation through the terminal capillary loops of the nailfold is interrupted by irregular, momentary cessations of blood flow which probably reflect periodic, irregular vasoconstriction of the contractile portions of the minute vascular bed. This process of vasomotion is usually intensified in patients with essential hypertension.

2. L-nor-epinephrine infused intravenously at increasing rates in persons with normal blood pressures induced progressively increasing vasomotion until the capillary bed resembled that seen in patients with essential hypertension. At this stage, the diastolic blood pressure increased an average of 11 mm. Hg but usually remained within normal limits.

3. Further increase in the rate of infusion of l-nor-epinephrine induced such marked vasomotion that the capillary loops became ischemic. Initial ischemia appeared and persisted when the diastolic blood pressure was elevated an average of 17 mm. Hg, although the diastolic blood pressure at this stage usually did not exceed 100 mm. Hg.

4. The capillary bed of patients with essential hypertension was hyper-reactive to circulating l-nor-epinephrine, as judged by the rates of infusion required to induce initial ischemia. Capillary ischemia appeared when the diastolic blood pressure was elevated by an average of only 4 mm. Hg.

5. The diastolic hypertensive states associated with essential hypertension and with the infusion of l-nor-epinephrine may have similar central systemic hemodynamics but do not have similar nailfold capillary circulations.

6. The alteration of the circulation in the capillary bed of the nailfold was a more sensitive index for detecting small quantities of intravenously injected l-nor-epinephrine than was the alteration of the arterial blood pressure, as determined by the auscultatory method.

7. The results and conclusions apply only to acute changes produced in the circulation through the capillary bed of the nailfold and in the systemic blood pressure by the intravenous infusion of l-nor-epinephrine.

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THE PLASMA "AMMONIA" AND GLUTAMINE CONTENT IN PATIENTS WITH HEPATIC COMA[1]

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Although the clinical state of hepatic coma is well recognized, it has not been associated with any consistent organic lesion of the central nervous system. This has led to the postulation of a biochemical defect to account for the impairment of the central nervous system observed in hepatic coma; however the biochemical nature and the ultimate cause of hepatic coma has not yet been found (1, 2). Certain nitrogenous substances given to patients with cirrhosis of the liver have produced a clinical syndrome indistinguishable from spontaneous impending hepatic coma (3, 4). These observations suggested that the quantitative determination of substances closely related metabolically to ammonia might provide a better biochemical correlation with the clinical state.

Since glutamine normally comprises fifteen to twenty-five per cent of the total α-amino acids of plasma (5) and may be formed metabolically by the combination of glutamic acid and ammonia (6) the plasma concentration of glutamine might reflect some aspects of ammonia metabolism. Using paper chromatography, Walshe (7) has shown that patients in hepatic coma may show increased amounts of glutamine along with other amino acids in urine and plasma and that the glutamine content of cerebrospinal fluid may be elevated.

Quantitative determinations of volatile base ("ammonia"), glutamine, and total α-amino nitrogen were performed on the plasma of normal adults and of patients with severe cirrhosis of the liver, impending hepatic coma, and hepatic coma. The effect of ammonium chloride administered intravenously on plasma glutamine was also determined.

METHODS AND MATERIALS

Patients were selected from the wards of the Boston City Hospital and the Metabolic Ward of the Thorndike Memorial Laboratory. Eleven laboratory personnel served as normal controls. The ten patients with cirrhosis were chronic alcoholics who had clinical as well as laboratory evidence of chronic liver disease, *i.e.*, spider angiomata, palmar erythema, palpable liver or spleen, ascites and edema, bromsulfalein retention, serum bilirubin elevation, and abnormal thymol turbidity and flocculation tests. Although oliguria was frequently observed in patients with coma, renal function as judged by azotemia was usually normal except terminally. (see Figures 2 and 3). Those thirteen patients classed as impending hepatic coma showed in addition mental confusion, lethargy, and the characteristic tremor described by Adams and Foley (8). "Semicoma" refers to a state of unconsciousness from which patients still could be aroused. Thirteen patients who were comatose generally could not be aroused even by painful stimuli, and often corneal reflexes were absent. Whenever possible, serial observations were made.

METHODS

Plasma collection. Venous blood was collected with precautions described by Conway and Cooke (9) without stasis in an oiled syringe and transferred immediately to a CO_2-filled test tube containing dried heparin (0.03 mg. per cc. blood) and chilled immediately in an ice bath. Plasma was separated by centrifugation in the cold and either assayed immediately or stored in a deep freeze at −21° C. Such storage did not change the plasma glutamine content for at least sixty days. In addition protein-free filtrates of plasma were made without delay after separation and stored in the deep freeze until subsequent analysis for α-amino nitrogen.

Assays. Glutamine was determined enzymatically utilizing the specific glutaminase of *Clostridium welchii* SR-12 (American Type Culture Collection No. 6784) by

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[2] On assignment from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service.

adapting the method of Krebs (10) to use with plasma. The organisms were grown, harvested and washed as described by Meister, Sober, and Tice (11), and suspended in 0.2 N acetate buffer pH 4.9 to give a total cellular nitrogen content of 4.6 mg. per cc. The preparation was stored in 25 cc. aliquots at − 21° C. and the aliquot in current use was stored at 4° C. Plasma was incubated with and without enzyme and the amide nitrogen liberated as ammonia from glutamine by glutaminase action was determined by a modification of the microdiffusion method of Seligson and Seligson (12). Standard glutamine and blanks for enzyme, glutamine and reagents were run with each set of determinations with the assays accounting for ninety-five to one hundred per cent of the ammonia expected from the total nitrogen content of a standard glutamine solution. Ninety-four per cent recovery of added glutamine was obtained from normal plasma following the addition of 12.5 μg. glutamine amide-N per cc. of plasma analyzed. Recovery from abnormal sera was not determined. All determinations were done in duplicate with a maximum difference of 0.4 μg. of nitrogen.

For the glutamine assays 5 cc. of plasma was adjusted to pH 4.8–5.0 with 4 N acetic acid using a Beckman pH meter. One cc. of plasma mixed with 1 cc. of 0.2 N acetate buffer pH 4.9 served as plasma blanks. For glutaminase treatment, 1 cc. plasma was mixed with 0.5 cc. 0.2 N acetate buffer pH 4.9 and 0.5 cc. of *Clostridium welchii* suspension described above. These mixtures were prepared in 20 cc. serum bottles. After a 30-minute incubation in a 38° C. water bath, the ammonia was liberated from solution by addition of 1 cc. of saturated potassium carbonate and collected by microdiffusion into a centrally suspended drop of phosphoric acid. The acid coated a 6 mm. spherical enlargement at one end of a 4 mm. glass rod suspended in the center of the bottle from a one-hole rubber stopper. The rods were stored with the ball portion immersed in 85 per cent orthophosphoric acid and were rinsed thoroughly with distilled water and only the rod portion was dried with a wipette immediately before use. This treatment permitted a uniform liquid film to be left after dipping the ball portion into 1 M phosphoric acid. After microdiffusion for 1 hour with horizontal rotation of the bottles at 34 r.p.m. and 23° C., the glass rods were removed and the ball portions rinsed thoroughly in 5 cc. of distilled water in a Coleman cuvette. One-half cc. of Nessler's solution prepared by the method of Vanselow (13) was added with mixing and the optical density at 500 mμ determined after 20 minutes with a Coleman Jr. Spectrophotometer and compared with simultaneously run ammonium sulfate standards. The plasma blank was used as an estimation of

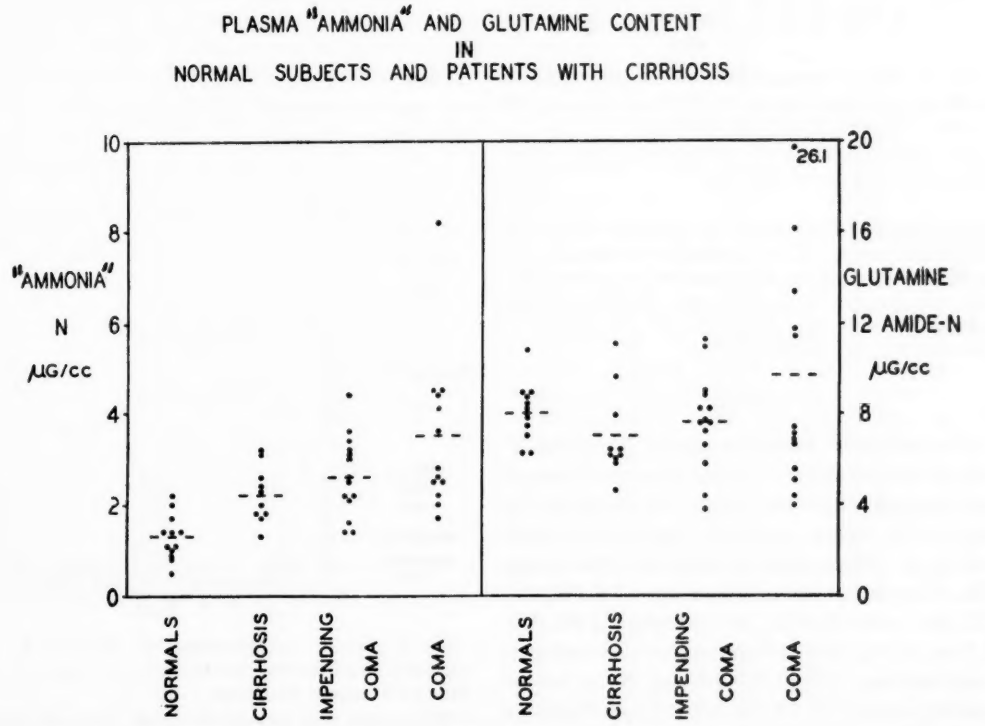

FIGURE 1

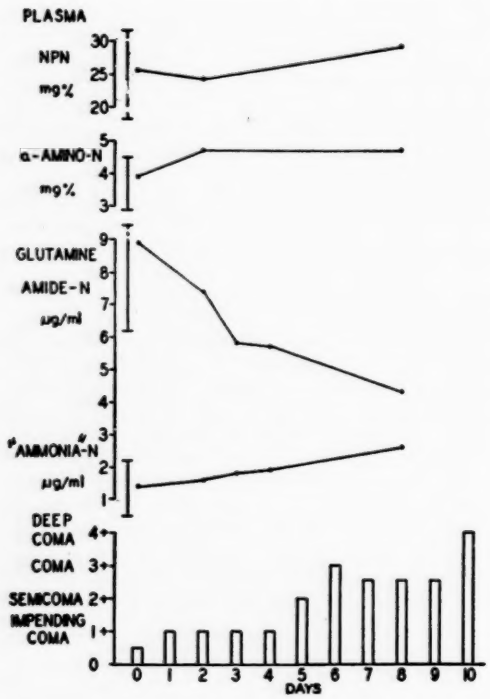

FIG. 2. SERIAL OBSERVATIONS ON PATIENT A. D. SHOWING THE RELATIONSHIP OF DEPTH OF COMA TO THE PLASMA NITROGEN FRACTIONS MEASURED

The vertical lines indicate the range of normal values. The patient died on the tenth day.

volatile plasma base indicated as "ammonia" in the accompanying charts. This is probably not true ammonia as the values for normal individuals are somewhat higher than are reported in the literature (9, 14). Plasma α-amino nitrogen was determined by the method of Hamilton and Van Slyke (15).

RESULTS

The individual values for plasma "ammonia-N" and glutamine amide-N found in normal controls and patients with liver disease are given in Figure 1. The plasma "ammonia" values show a definite trend toward high values in the coma group. The mean and range of values are : 1.3 (R. 0.5–2.2) μg. ammonia-N/cc. for normals, 2.2 (R. 1.3–3.2)μg. N/cc. for cirrhosis without neurological complications, 2.6 (R. 1.4–4.4) μg. N/cc. for impending coma, and 3.5 (R. 1.7–8.2) μg. N/cc. for coma. The scatter of values about the mean for the coma group indicates that half the values are no different from the values found in cirrhosis without neurological complications. The individual values do not consistently correlate with the neurological status.

The glutamine values indicate considerable variation. The mean and range of plasma glutamine values are: 8.04 (R. 6.2–10.8) μg. amide-N/cc. for normal subjects, 7.02 (R. 4.6–11.1) for patients with cirrhosis, 7.66 (R. 3.8–11.3) for patients with cirrhosis with impending coma, and 9.7 (R. 4.0–26.1) for patients with hepatic coma. The patients with impending hepatic coma show values no different from those without neurological complications. Of the thirteen patients in coma, while five had initial glutamine values greater than the

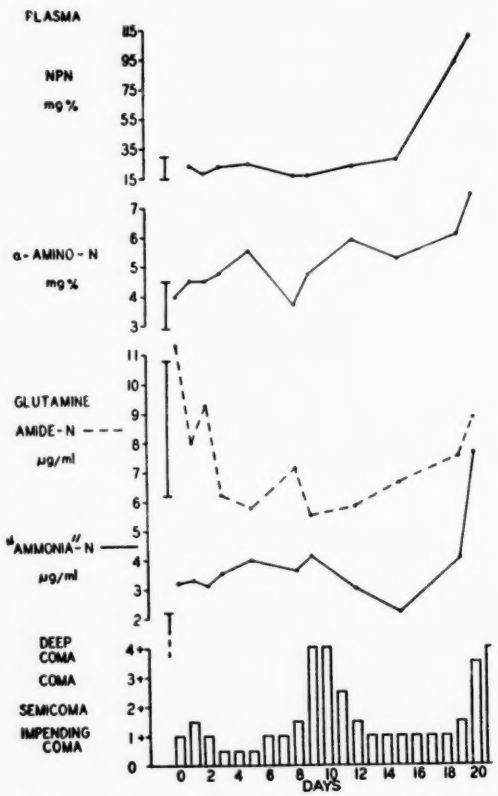

FIG. 3. SERIAL OBSERVATIONS ON PATIENT T. H. SHOWING THE RELATIONSHIP OF DEPTH OF COMA TO THE PLASMA NITROGEN FRACTIONS

The vertical lines indicate the range of normal values. The patient died on twenty-first day of observation.

normal range, the majority of patients had normal values.

Serial observations have revealed no correlation between neurological status and any fraction of the plasma non-protein nitrogen measured. Two patients followed serially are presented in Figures 2 and 3. The plasma glutamine data are within the normal range throughout, although in both cases the plasma alpha amino-nitrogen fraction is elevated. In two other patients with coma, elevated plasma α-amino N was noted (values 5.4 and 8.1 mg. per cent, the normal range being 2.9–4.5 mg. per cent) with plasma glutamine concentrations of 6.7 and 7.1 μg. amide N/cc., respectively, which are within the normal range. There is a suggestive correlation of plasma "ammonia-N" and depth of coma in case 2 (Figure 3).

The relationship of plasma (or blood) "ammonia" concentration and neurological status was further evaluated by the intravenous administration of 1 or 2 per cent ammonium chloride in distilled water to four patients with alcoholic cirrhosis of the liver without evidence of impending coma. In six infusions, at a maximal rate of 1100 cc. of 2 per cent solution in 2½ hours, blood or plasma levels as great as 18 μg. NH_3–N/cc. (1.8 mg. per cent) were achieved. The characteristic "flapping" tremor and mental confusion of impending coma were observed in only one patient (Figure 4). All patients developed nausea and vomiting at rapid infusion rates (greater than 60 drops per min. of 2 per cent solution). In two cases serial measurements of plasma glutamine indicated stable concentrations throughout although plasma "ammonia" values reached peak levels of 13.6 and 18.4 μg. ammonia–N/cc. during the infusion.

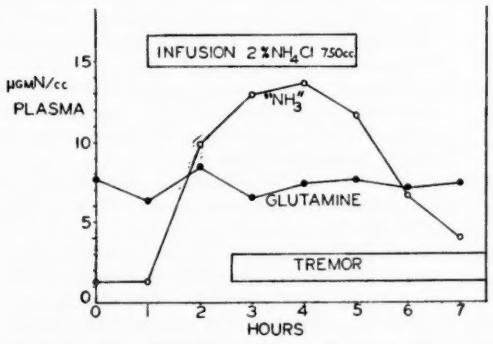

FIG. 4. THE RELATIONSHIP OF PLASMA GLUTAMINE TO "AMMONIA" DURING THE INFUSION OF 2 PER CENT AMMONIUM CHLORIDE TO A PATIENT WITH CIRRHOSIS, SHOWING ALSO THE DEVELOPMENT OF THE CHARACTERISTIC TREMOR OF IMPENDING COMA

DISCUSSION

Studies of ammonia metabolism by Foster, Schoenheimer, and Rittenberg (16) using N^{15} labeled ammonium ion in the rat have shown that the most rapid incorporation was into an amide nitrogen fraction which is presumably the amide group of glutamine. Incorporation into the α-amino nitrogen of glutamic and aspartic acids also occurred but at a much slower rate. This seems to indicate a close metabolic relationship between ammonia metabolism and glutamine, although enzyme studies (17) have not placed glutamine in the direct pathway of reactions leading to the formation of urea which is the main end product of metabolized ammonia. Glutamine formation from ammonia and glutamic acid has been proposed as a biological method for detoxification and transport of ammonia (18, 19). A role of metabolic buffer for providing Krebs cycle intermediates has also been proposed (20) and recently evidence for a fundamental role in transfer reactions leading to peptide synthesis has been found (21).

Quantitative determinations of plasma glutamine were made to evaluate the possibility that alterations in plasma glutamine concentration might reflect altered ammonia metabolism. The results do not support this concept. However, it is quite possible that the tissue content of glutamine could be affected by the abnormalities in ammonia metabolism without affecting plasma levels. In hepatectomized dogs, an increased content of glutamine in brain, muscle and plasma has been observed (22). The glutamine content of brain was not proportional to the plasma content.

The failure to observe any relationship between clinical status and plasma glutamine content does not indicate that glutamine-ammonia metabolism is unrelated to the neurological status. Although a better correlation has been observed between plasma "ammonia" and the clinical status, the ammonium infusion studies suggest that a direct quantitative and temporal relationship does not exist. Even though the plasma content of am-

monia was elevated for several (2 to 4) hours, neurological abnormalities were observed in only one patient.

SUMMARY

The specific glutaminase of *Cl. Welchii* has been adapted for quantitative analysis of plasma glutamine. Plasma from normal adults and patients with cirrhosis of the liver was assayed for the content of glutamine and volatile base (ammonia) liberated during microdiffusion. The values for plasma glutamine were similar in all groups evaluated. The values for volatile base, "ammonia," tended to be elevated in the impending hepatic coma and hepatic coma groups compared to the controls.

No correlation between plasma glutamine and neurological status of the patients with cirrhosis was noted in individual patients observed serially or in the groups as a whole. A suggestive correlation of neurological status and the plasma "ammonia" is evident in the mean values for the groups; however, this was not consistently present in serial measurements in individual patients.

The characteristic neurological findings of impending hepatic coma were produced in one of three patients in whom elevated plasma "ammonia" levels were produced by the infusion of ammonium chloride without any effect on the concentration of plasma glutamine.

ACKNOWLEDGMENT

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BODY WATER AND SODIUM IN PATIENTS WITH ACROMEGALY [1]

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The acromegalic state often is accompanied by an enlargement of the soft tissues of the body. The purpose of the present work was to determine whether these tissue changes are accompanied by changes in the content and distribution of water and electrolytes in the body. In this connection the administration of growth hormone was shown to induce an increase in the total body water of normal (1–4) and hypophysectomized (5) rats, and of normal dogs and cats (3). Similarly, growth hormone caused a retention of sodium and potassium in normal (6) and diabetic (7) rats, and a retention of sodium, potassium, and chloride in adrenalectomized rats (8). The thiocyanate space in normal and hypophysectomized rats (9) and the plasma volume in dogs (10) increased after injection of growth hormone.

The present paper gives the results of measurements of the total body water, the extracellular water and the exchangeable sodium in 18 acromegalic patients.

MATERIAL AND METHODS

Total body water was calculated from the volume of distribution of antipyrine (11). Total exchangeable sodium was calculated from the apparent volume of distribution of the radioactive sodium isotope Na^{24} (12). The extracellular water was calculated from the volumes of distribution of inulin (13) or thiosulfate (14). In six cases, both of these methods were used to estimate the volume of extracellular water. In six cases, no measurement of the extracellular fluid was made.

All experiments started at 8 a.m. The patients were not allowed to eat after 8 p.m. the day before the test. Urine was collected the preceding day for the determination of the blank excretion of inulin. The antipyrine was given from a calibrated burette, and venous blood samples were drawn after two, three, four, and five hours. Immediately after the injection of antipyrine the constant infusion of inulin was started. The infusion of inulin was continued for 6 to 7 hours. When simultaneous measurements of the thiosulfate and inulin spaces were performed, thiosulfate was injected immediately after the inulin test was completed. If the thiosulfate space only was used for the measurement of the extracellular water, the test was performed the day after the determination of total body water, since thiosulfate interferes with the chemical analysis of antipyrine in plasma.

Radioactive sodium, Na^{24}, was injected intravenously, immediately after the determination of the extracellular water was completed. Urine was collected for the following 18 hours, and blood samples were taken at the end of the urine collection period.

Antipyrine in plasma was analyzed according to Brodie, Axelrod, Soberman, and Levy (15). In our hands the time necessary for the completion of the reaction between antipyrine and sodium nitrite was different from day to day. Repeated readings, therefore, were taken every five to ten minutes after the addition of sodium nitrite. The maximal extinctions obtained were plotted on a semilogarithmic paper, and the extinction at zero time was obtained by extrapolation. The volume of distribution of antipyrine was calculated by dividing the given amount of antipyrine by its zero time plasma concentration.

The inulin in plasma was analyzed by Roe's resorcinol method, as modified by Schreiner (16). The inulin space was obtained by dividing the total amount of inulin excreted after discontinuation of the infusion by the inulin concentration in plasma at equilibrium. The thiosulfate in plasma was analyzed by the indirect iodometric-macromethod of Newman, Gilman, and Philips (17). The thiosulfate concentration at zero time was obtained by extrapolation, and the volume of distribution calculated from this value and the amount of thiosulfate given.

The concentration of sodium in plasma was measured by a Beckman flame photometer.

The conversion of the antipyrine, inulin, and thiosulfate spaces to total body water and extracellular water, respectively, was made by multiplying the values obtained by 0.93. The concentration of sodium in plasma water was calculated from the corresponding value in plasma, using the same figure, 0.93. The plasma water content of the acromegalic patients was taken as normal.

The surface area of the body was derived from the nomogram of DuBois, although the authors are aware of the fact that the applicability in acromegaly of the DuBois surface area tables has not been proved.

Intracellular water was taken as the difference between total body water and extracellular water. Total ex-

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TABLE I

Case material

Case no.	Patient	Age *years*	Sex	Height *cm.*	Weight *Kg.*	Surface area m^2	Duration of disease *years*	Soft tissue swelling	Plasma phosphorus, *mg. %*		
									At the present study	Highest value observed*	Lowest value observed*
1	S. K.	36	M	179	87.6	2.04	1	+	3.4	4.8	3.4
2	M. H.	53	F	164	68.0	1.73	10	−	4.0		
3	D. P.	35	F	177	76.0	1.94	5	+	4.6		
4	C. B.	40	M	181	101.0	2.20	10	+	3.9	3.9	2.9
5	E. E.	23	F	169	75.6	1.85	3	+	4.4		
6	A. A.	43	F	156	63.0	1.61	10	−	3.7	4.7	3.7
7	A. F.	42	M	195	99.0	2.31	10	+	5.2		
8	J. I.	60	M	175	80.8	1.95	30	+	3.6	4.6	3.6
9	L. H.	23	M	199	92.0	2.26	2	−	5.1	5.1	4.3
10	E. J.	40	F	165	74.0	1.81	3	−	3.3	4.7	3.3
11	M. M.	42	F	167	75.0	1.83	5	−	3.4	4.7	3.3
12	C. L.	20	M	197	95.0	2.28	2	+	5.6	5.6	4.4
13	S. A.	49	F	174	78.0	1.92	7	+	4.4		
14	H. W.	51	M	175	80.0	1.95	15	+	3.4	4.0	3.4
15	K. L.	52	M	178	89.7	2.08	5	+	4.1	5.3	3.4
16	M. S.	32	F	173	78.0	1.90	5	+	5.8	5.8	4.3
17	I. B.	30	M	177	78.1	1.94	5	+	5.2	5.4	4.7
18	F. O.	38	M	182	92.5	2.12	10	+	4.5	4.5	3.9

* These values were observed during the whole period of observation.

changeable sodium (mEq.) was calculated from the apparent volume of distribution of radio-sodium Na^{24} (liters) and the concentration of sodium in the plasma water (mEq. per liter). Extracellular sodium was calculated as the product of plasma water sodium concentration (mEq. per liter) and extracellular water volume (liters). Intracellular sodium was calculated as the difference between total body sodium and extracellular sodium. The apparent mean intracellular sodium concentration (mEq. per liter) was calculated by dividing the intracellular sodium by the intracellular water volume.

The material of the present study consisted of 18 cases

TABLE II

Total body water and total exchangeable sodium in patients with acromegaly

Case no.	Total body water			Sodium *mEq./L.* plasma water	Total exchangeable sodium			
	L.	% of body weight	*L./m²* surface area		*mEq.*	*mEq./Kg.* body weight	*mEq./m²* surface area	*mEq./L.* total body water
1	49.6	56.6	24.3	154	4,620	52.7	2,265	93.1
2	30.7	45.1	17.8	151	3,956	58.2	2,287	128.8
3	41.0	52.7	21.1	148	4,248	54.6	2,190	103.6
4	51.2	50.7	23.3	154	5,452	54.0	2,478	106.4
5	34.0	45.0	18.4	151	3,639	48.1	1,967	107.0
6	34.0	54.0	21.1	151	3,413	54.2	2,120	100.4
7	63.1	63.7	27.3	152	6,202	69.7	2,685	98.3
8	45.2	55.9	23.2	151	4,651	57.6	2,385	102.9
9	54.8	59.6	24.2	154	4,420	48.0	1,956	80.7
10	43.0	60.8	24.9	152	3,587	48.5	1,982	79.7
11	35.8	47.7	19.6	154	3,003	40.0	1,641	83.9
12	60.6	63.8	26.6	151	5,527	58.2	2,424	91.2
13	40.1	51.4	20.9	153	3,917	50.2	2,040	97.7
14	45.0	56.2	23.1	153	4,712	58.9	2,416	104.7
15	55.5	61.9	26.7	154	4,404	49.1	2,117	79.4
16	43.7	56.2	23.0	154	3,788	48.6	1,994	86.7
17	43.6	55.8	22.5	151	3,700	47.4	1,907	84.8
18	62.9	68.0	29.7	149	4,843	52.4	2,284	77.0
Average		55.8	23.2			52.8	2,174	94.8
Standard error of mean		1.5	0.7			1.5	60	3.1
Healthy subjects:								
Average		49.7	18.6			37.1	1,386	74.7
Standard error of mean		0.9	0.5			0.9	35	1.4
Significance of difference:								
P-value		<0.01	<0.001			<0.001	<0.001	<0.001

TABLE III

Distribution of water and sodium in patients with acromegaly (extracellular water calculated from the inulin space)

Case no.	Extracellular water (ECW)				Intracellular water (ICW)				Ratio ICW:ECW	Exchangeable sodium (Na_e)					Ratio IC:EC
										Extracellular (EC)		Intracellular (IC)			
	L.	% of body weight	*L./m²* surface area	% of total body water	*L.*	% of body weight	*L./m²* surface area	% of total body water		*mEq.*	% of total Na_e	*mEq.*	% of total Na_e	*mEq./L.* ICW	
1	15.5	18.2	7.7	29.9	36.4	42.7	18.0	70.1	2.35	2,294	47.8	2,501	52.2	68.7	1.09
6	13.5	17.3	7.0	33.7	26.6	34.1	13.9	66.3	1.97	2,066	52.7	1,851	47.3	69.6	0.90
7	24.8	25.1	10.7	39.3	38.3	38.7	16.6	60.7	1.54	3,770	60.7	2,432	39.2	63.5	0.65
8	19.1	23.6	9.8	42.3	26.1	32.3	13.4	57.7	1.37	2,884	62.0	1,767	38.0	67.7	0.61
9	18.5	20.1	8.2	33.8	36.3	39.5	16.1	66.2	1.96	2,849	64.5	1,571	35.5	43.2	0.55
11	10.1	13.5	5.5	28.2	25.7	34.3	14.0	71.8	2.54	1,525	51.8	1,420	48.2	55.2	0.93
15	17.8	18.7	7.8	29.4	42.8	45.0	18.8	70.6	2.40	2,689	48.7	2,838	51.3	66.3	1.06

TABLE IV

Distribution of water and sodium in patients with acromegaly (extracellular water calculated from the thiosulfate space)

Case no.	Extracellular water (ECW)				Intracellular water (ICW)				Ratio ICW:ECW	Exchangeable sodium (Na_e)					Ratio IC:EC
										Extracellular (EC)		Intracellular (IC)			
	L.	% of body weight	*L./m²* surface area	% of total body water	*L.*	% of body weight	*L./m²* surface area	% of total body water		*mEq.*	% of total Na_e	*mEq.*	% of total Na_e	*mEq./L.* ICW	
1	22.0	25.1	10.8	44.4	27.6	31.5	13.5	55.6	1.3	3,388	73.3	1,240	26.8	44.9	0.37
2	11.0	16.1	6.4	35.8	19.7	29.0	11.4	64.2	1.8	1,661	42.0	2,295	58.0	116.5	1.38
3	15.8	20.3	8.1	38.5	25.2	32.4	13.0	61.5	1.6	2,338	55.0	1,910	45.0	75.8	0.82
4	19.3	19.1	8.8	37.7	31.9	31.6	14.5	62.3	1.7	2,972	54.5	2,480	45.5	77.7	0.83
5	14.9	19.7	8.1	43.8	19.1	25.3	10.3	56.2	1.3	2,250	61.8	1,389	38.2	72.7	0.62
6	13.4	21.3	8.3	39.4	20.6	32.7	12.8	60.6	1.5	2,023	59.3	1,390	40.7	67.5	0.69
7	26.0	26.3	11.3	41.2	37.1	37.5	16.1	58.8	1.4	3,952	63.7	2,250	36.3	60.6	0.57
8	17.2	21.3	8.8	38.1	28.0	34.7	14.4	62.0	1.6	2,597	55.8	2,054	44.2	73.4	0.79
9	19.9	21.6	8.8	36.3	34.9	37.9	15.4	63.7	1.8	3,065	69.3	1,355	30.7	38.8	0.44
10	14.3	19.3	7.9	31.7	31.7	41.5	16.9	68.2	2.1	2,174	60.6	1,413	39.4	46.0	0.65
11	11.1	14.8	6.1	31.0	24.7	32.9	13.5	69.0	2.2	1,676	56.9	1,269	43.0	51.4	0.76

of acromegaly (Table I). In none of the patients thyrotoxicosis, diabetes mellitus, testicular atrophy or adrenal cortical insufficiency could be demonstrated. Signs of congestive heart failure were not present in any of the cases. No instances of acromegalic gigantism appeared in these patients. The cases were unselected with respect to the activity of the disease; cases Nos. 6, 11, and 15 were therefore included despite the fact that they were considered almost "cured." The table gives clinical and laboratory data, describing the status of the patients at the time of the present study.

RESULTS

In the acromegalics (Table II) the average total body water was found to be 55.8 per cent of the body weight or 23.2 liters per square meter of body surface. The corresponding figures for the normal group of nine healthy subjects (18) were 49.7 per cent and 18.6 liters, respectively. The differences between the figures of the two groups were statistically significant.

The amount of total exchangeable sodium in the acromegalics corresponded to 52.8 mEq. per Kg. body weight or 2174 mEq. per square meter of surface area or 94.8 mEq. per liter of total body water. These figures were significantly higher than the corresponding ones in the group of healthy subjects.

The distribution of body water between the intra- and extracellular phases, using the inulin or thiosulfate spaces in the calculation of the amount of extracellular water, is presented in Tables III and IV, respectively. In Tables III and IV is also presented the distribution of exchangeable sodium between the intra- and extracellular spaces using inulin or thiosulfate. The mean values of the results presented in Tables III and IV are given in Table V together with the corresponding figures obtained by similar measurements in the group of healthy subjects (18). Table V shows that extracellular water expressed as liters per square meter of surface area or as percentage of body weight was significantly increased in the acromegalics, whether the calculations were made from the inulin or thiosulfate spaces. However, when expressed as percentage of total body water, the extracellular water was significantly higher, only when the thiosulfate space was used.

The amount of intracellular water expressed as per cent of body weight was of the same magnitude in the acromegalics and in the healthy subjects. Expressed as liters per square meter of surface area it was different (higher) in the acromegalics, only when calculated from the inulin space. When expressed as per cent of total body water it was significantly higher in the acromegalics, only when calculated from the thiosulfate space.

The distribution of exchangeable sodium between the intra- and extracellular fluid compartments in the acromegalics did not differ from that in the healthy subjects. However, the "apparent" mean intracellular sodium concentration

TABLE V

A comparison of the distribution of water and exchangeable sodium in healthy subjects and acromegalics

	Body water							Exchangeable sodium (Na_e)			
	Extracellular (ECW)			Intracellular (ICW)				Extracellular (EC)	Intracellular (IC)		
	% of body weight	*L./m²* surface area	% of total body water	% of body weight	*L./m²* surface area	% of total body water	Ratio ICW:ECW	% of total Na_e	% of total Na_e	*mEq./L.* ICW	Ratio IC:EC
Extracellular water calculated from the *inulin* space											
Normals (9 subjects)											
Average	14.8	5.5	29.9	34.9	13.0	70.1	2.4	60.3	39.7	42.4	0.67
S.E.*	0.3	0.2	0.7	0.9	0.4	0.7	0.1	1.5	1.5	2.0	0.05
Acromegaly (7 subjects)											
Average	19.5	8.1	33.8	38.1	15.8	66.2	2.0	55.5	44.5	62.0	0.83
S.E.*	1.5	0.7	2.0	1.8	0.8	2.0	0.2	2.6	2.6	3.6	0.08
P-value	<0.01	<0.01			<0.01					<0.001	
Extracellular water calculated from the *thiosulfate* space											
Normals (9 subjects)											
Average	15.2	5.7	30.5	34.6	12.9	69.5	2.3	61.2	38.8	41.7	0.64
S.E.*	0.3	0.2	0.8	0.9	0.4	0.8	0.1	1.4	1.4	1.7	0.04
Acromegaly (11 subjects)											
Average	20.5	8.5	38.0	33.4	13.8	62.0	1.7	59.3	40.7	65.9	0.72
S.E.*	1.0	0.5	1.3	1.3	0.6	1.3	0.1	2.5	2.5	6.6	0.08
P-value	<0.001	<0.001	<0.001			<0.001	<0.001			<0.01	

* Standard error of mean.

was significantly higher in the acromegalic patients.

DISCUSSION

The question may be raised, whether, with the methods used, it is possible to obtain exact measurements of the total body water and extracellular water.

The antipyrine space in healthy subjects has been found to be 2 to 5 per cent smaller than the deuterium or tritium space (11, 19, 20). The same relation was obtained in some diseased states (21) except in the presence of edema (22). This constancy shows that the antipyrine space represents at least a constant fraction of the total body water if not the true volume. In edematous patients the antipyrine space has been found to be about 15 per cent smaller than the deuterium space (22). No comparison has been made between the antipyrine and deuterium spaces in acromegaly. Whether the ratio of the antipyrine space to the deuterium space in acromegalics is smaller, as in edematous patients, or of the same magnitude as in healthy subjects, the significance of the differences found in the present work will still hold true.

It is very much discussed, whether the inulin and thiosulfate volumes of distribution represent the whole extracellular compartment. However, it has been shown that they measure a reproducible space, which is believed to be the functional extracellular fluid volume (23). Consequently, we have used the changes in the inulin and thiosulfate spaces as indices of changes in the extracellular compartment. "Extracellular water" in the present work therefore represents the portion of the true extracellular water, which is included in the inulin and thiosulfate space. From this it is obvious that the calculated "intracellular water" in the present work might be somewhat overestimated.

A better reference for the fluid compartments would perhaps be the correlation to the lean body mass. However, we did not consider the formulas developed for the calculation of the lean body mass *a priori* applicable to cases of acromegaly, where nothing is known about the mean water content of the lean body mass or the cell mass. In view of the abnormal body build in acromegaly we have correlated our results to body weight, surface area, and in the case of extracellular and intracellular water and exchangeable sodium also to total body water.

The finding of an increased total body water content in acromegalics is of interest in the light of the results obtained in the animal experiments mentioned in the introduction, as an abnormal secretion of growth hormone is generally believed to be, if not the only, at least the principal factor in the production of the clinical picture of acromegaly.

The increase in extracellular water in the acromegalics, when expressed as percentage of body weight or as liters per square meter of surface area, obtained with inulin as well as with thiosulfate, two substances so different in many respects, favors the view that the observed change in the extracellular water volume was a true increase and not due to alterations of the permeability of the cell membranes. Although the latter possibility cannot be excluded, the large increase in exchangeable sodium strongly favors the view that the extracellular space was increased.

The extracellular water represented a larger part of the total body water in the acromegalic than in the healthy subjects, but the difference was statistically significant with thiosulfate only. In three (Nos. 1, 11, and 15) of the seven patients studied with inulin the inulin space formed an entirely normal percentage of the total body water. Two of these cases (Nos. 11 and 15) had been treated for a long time and were considered almost "cured." Our results favor the hypothesis that in acromegaly the extracellular water is increased disproportionally to the intracellular water.

The increase in total exchangeable sodium observed in acromegaly was concomitant with the increase in the extracellular space. As to the observed increase in the "apparent" mean intracellular sodium concentration this finding does not prove an actual increase in the sodium content of the cells. It is known that about fifty per cent of the body sodium is to be found in the skeleton and that only about half of it exchanges with radiosodium in healthy subjects (24). An increase in the percentage of the bone sodium exchanged or a disproportional increase in the bone mass over the soft tissue would give an increased "mean intracellular sodium concentration."

The significance of the changes observed in the acromegalics, *i.e.*, the increase in total body water and in total exchangeable sodium and the prob-

ably disproportional increase in the amount of extracellular water, cannot be decided at present. However, these differences between the acromegalic and healthy subjects are similar to those observed between infants and adults (19, 25, 26).

SUMMARY

The amount of total body water (antipyrine space) per Kg. of body weight and per square meter of body surface, was significantly increased in a group of 18 acromegalic patients.

The amount of total exchangeable sodium in mEq. per Kg. of body weight, per square meter of surface area or per liter of total body water was also increased in the same patients.

The amount of extracellular water (inulin or thiosulfate spaces) per Kg. of body weight or per square meter of surface area was higher in the acromegalic patients.

The apparent mean intracellular sodium concentration was higher in the acromegalic subjects.

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I. THE SEPARATION OF SODIUM FROM POTASSIUM IN HUMAN BLOOD SERUM BY ION EXCHANGE CHROMATOGRAPHY

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The experiments to be described were undertaken to devise a simple and rapid technique for the separation of sodium from potassium in human blood serum in order to facilitate the simultaneous measurement of exchangeable sodium and potassium in man by the isotope dilution technique. This technique requires the intravenous administration of a known amount of radioactive sodium (Na^{24}) and radioactive potassium (K^{42}), followed by the determination, after a twenty-four hour equilibration period, of the individual sodium and potassium radioactivities and concentrations in the serum. The general principles underlying the application of the isotope dilution technique to the measurement of body constituents have been outlined by Edelman, Olney, James, Brooks, and Moore (1). The data presented here are primarily concerned with the separation of sodium from potassium in aqueous solutions and serum. The results of our measurements in man are presented in Part II of this study (2).

Since the isotope dilution method for the measurement of exchangeable body sodium and potassium requires separate determinations of the radioactivity of the Na^{24} and K^{42} found in the serum, three methods for making the discrimination between sodium and potassium were considered: 1) Physical separation, which makes use of differences in the physical characteristics of the radiation from the two isotopes; 2) chemical separation by precipitation; and 3) chemical separation by ion exchange.

It is difficult at present to make accurate individual measurements based on differences in physical properties of the radioactivity of Na^{24} and K^{42} in mixtures of the two isotopes, because their half-lives, 15.06 and 12.44 hours, respectively, are very similar, and because both isotopes emit beta particles and gamma rays whose energies are not markedly different.[1]

Chemical separation by precipitation can be carried out by a standard procedure, and James, Brooks, Edelman, and Moore (4) have developed a technique for adapting such a procedure to the requirements of the isotope dilution method. We have adopted chemical separation by ion exchange, making use of the sulfonic acid cation exchange resin, Dowex 50, in order to simplify the separation for clinical use. This procedure depends upon the greater affinity of Dowex 50 for the potassium ion than for the sodium ion so that these alkali metals may be eluted separately from the resin column with hydrochloric acid.

For our purposes the separation procedure had to satisfy the following special criteria: speed and completeness of separation, relative simplicity, and acceptable reproducibility. The importance of speed is apparent from the following facts. The amount of isotope that can be safely administered to man for investigative purposes is closely limited, and consequently our dose does not exceed 0.45 rep (roentgen equivalent physical). Approximately two half-lives elapse in the 24-hour period between injection and removal of the blood sample, with a resultant four-fold decrease in radioactivity. Furthermore, the amount of radioactivity in the 50 ml. sample of blood drawn represents but a small fraction of the amount injected. Thus, it is essential to reduce to a minimum the elapsed time between drawing the blood sample and measuring

[1] The radiation from Na^{24} and K^{42} is given by Hollander, Perlman, and Seaborg (3) as:

Na^{24} β^-, 1.39 Mev. (~100%), 4.15 Mev. (0.003%); γ, 1.38 Mev. (~100%), 2.76 Mev. (~100%); 4.1 Mev. (0.04%)

K^{42} β^-, 3.58 Mev. (75%), 2.04 Mev. (25%); γ, 1.5 Mev. (25%)

the radioactivity in order to obtain a reasonable counting rate and accurate results.

The necessity for a complete separation arises from the disparate concentration of sodium and potassium in blood serum (143 mEq. Na per L. and 4.65 mEq. K per L.). Although the initial injection is adjusted so that twice as much K^{42} as Na^{24} is administered, a contamination of the potassium by 1 per cent of the sodium would result in a 15 per cent error in the K^{42} measurement. To obtain a cross-contamination of less than 1 per cent only 0.07 per cent of Na^{24} can be tolerated in the K^{42} fraction.

Since Cohn and Kohn (5) first demonstrated that a sulfonated cation exchange resin, Dowex 50, could be used to separate the alkali cations, a number of other investigators, Kayas (6), Beukenkamp and Rieman (7), and Sweet, Rieman, and Beukenkamp (8), have investigated the use of resins to separate sodium from potassium. None of the procedures developed by these workers fully satisfied the criteria we had formulated. The separations of Cohn and Kohn and Kayas required over 24 hours, while the method of Beukenkamp and Rieman required the use of a column almost 60 cm. long, composed of colloidal Dowex 50, a mesh size which does not give reproducible results from batch to batch. Consequently, it was decided to determine by further studies with Dowex 50 the optimum combinations of such variables as mesh size, column length, cross linkage, normality of eluting acid, temperature, and rate of flow for our special needs.

APPARATUS AND REAGENTS

The resin was procured from the Dow Chemical Company [2] in the hydrogen cycle. To insure complete freedom of the resin from any small residual content of sodium and potassium, the resin was first eluted in column form with 300 ml. of 3 N hydrochloric acid at a rate of 1.5 ml. per minute. It was then washed with distilled water until the effluent was neutral to litmus paper. The resin columns rested on a glass wool plug or on a sintered glass filter (medium porosity) in a glass column of 1.3 to 1.6 cm. diameter. The wet resin was poured into the column and allowed to settle to the desired height. A small loose plug of glass wool was then placed on top of the resin column.

[2] We wish to express our appreciation to the Dow Chemical Co., Midland, Michigan, for their extremely helpful cooperation and for providing much of the resin used in these studies.

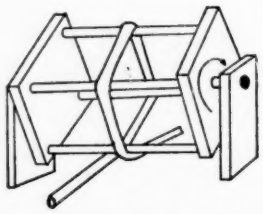

FIG. 1. CONSTANT FLOW PUMP

The rubber tubing is pulled tightly over the four cross bars in the pump. Each of the cross bars can turn on its long axis as the pump rotates. The volume of eluate delivered is determined by the diameter of the rubber tubing and the speed with which the pump revolves. Four tubes can be wound around the pump in parallel to deliver at equal rates.

Hydrochloric acid (C.P.) was used as the eluting agent (elutriant). Samples were collected in the tubes of a fraction collector and the rate of flow was maintained at a constant speed with the aid of an electrically driven pump [3] (Figure 1) introduced into the system between the column and the fraction collector. The pump consisted of four 0.6 cm. round stainless steel bars spaced at equal intervals on a circle of 3.7 cm. radius around a central shaft. Gum rubber tubing with an inside diameter of 2 to 3 mm. was tightly wound around the steel cross-bars, which were themselves free to rotate around their long axes. As the pump rotated the fluid was propelled through the rubber tubing by the pressure of the bars.

Measurements of sodium and potassium concentration in the effluent were made with the use of the radioactive isotopes Na^{24} and K^{42} obtained from the Atomic Energy Commission at the Brookhaven National Laboratory. The radioactivity was measured by use of a Geiger-Mueller dip counter described by Solomon and Estes (9) mounted in a special stand designed by Dr. C. V. Robinson to accommodate 8 ml. samples.

EXPERIMENTAL PROCEDURE

The test solution was an aqueous mixture of sodium chloride with a concentration of 140 mEq. per L. and potassium chloride with a concentration of 4.5 mEq. per L. containing approximately 10^6 counts per min. of Na^{24} and the same amount of K^{42}. These concentrations were chosen to simulate human serum concentrations. With our counting equipment and its background of 20 to 30 counts per minute we could easily obtain reliable measurements of activities of 100 counts per minute so that 0.01 per cent of the material added to the column could be detected.

Ten to forty ml. of test solution were added to the resin column at rates up to 1.5 ml. per minute. Eight

[3] We wish to express our appreciation to Ionics, Inc., Cambridge, Massachusetts, for their kindness in suggesting the use of such a pump and in making its design available to us.

hundred ml. of eluting acid were then run through the column at rates varying from 0.74 to 1.1 ml. per min. and collected in 10 ml. fractions. The radioactivities of these fractions were measured in the dip counter. For this purpose one minute counts which gave probable errors of up to 5 per cent provided satisfactory accuracy. By calculating the per cent of added radioactivity in each of these tubes elution curves for Na^{24} and K^{42} could be drawn.

Although the test solution was an aqueous one, equivalent results have also been obtained in the separation of sodium from potassium in serum. It was found experimentally that serum could not be added to the column directly without prior protein precipitation. For these experiments, 10^6 counts per min. of Na^{24} and K^{42} were added to 20 ml. of serum; the protein was then precipitated with 15 ml. of 10 per cent trichloracetic acid (C.P.), centrifuged at 1280 G and washed with an additional 10 ml. of reagent. After the addition of the supernatant and the washings to the resin column, approximately 10 per cent of the added Na^{24} and K^{42} remained absorbed on the precipitate which was not a significant loss of radioactivity for counting purposes.

RESULTS

Dowex 50 mesh sizes, 200 to 400, minus 325 (200 to 400 screened through a 325 mesh screen), minus 400, and colloidal agglomerates were investigated. Although the colloidal agglomerates theoretically (10) would provide the optimum mesh size for the separation, this resin size was not adopted since Sweet, Rieman, and Beukenkamp (8) had found the performance of colloidal agglomerates relatively non-reproducible from batch to batch. Consequently, we chose minus 325 mesh and, when it later became available, minus 400 mesh.

Figure 2 demonstrates the variation in the elution curves of sodium and potassium resulting from increasing the cross-linkage of the resin from 8 per cent to 12 per cent. Twelve per cent cross-linkage was determined to be the most favorable for our purpose.

The effect of varying the length of the resin column on the separation of the sodium and potassium ions is demonstrated in Table Ia. This table shows that an increase in length of the column by 0.5 cm. under these conditions very effectively drew the Na^{24} and K^{42} peaks apart. While a long column assured a complete separation, it also increased

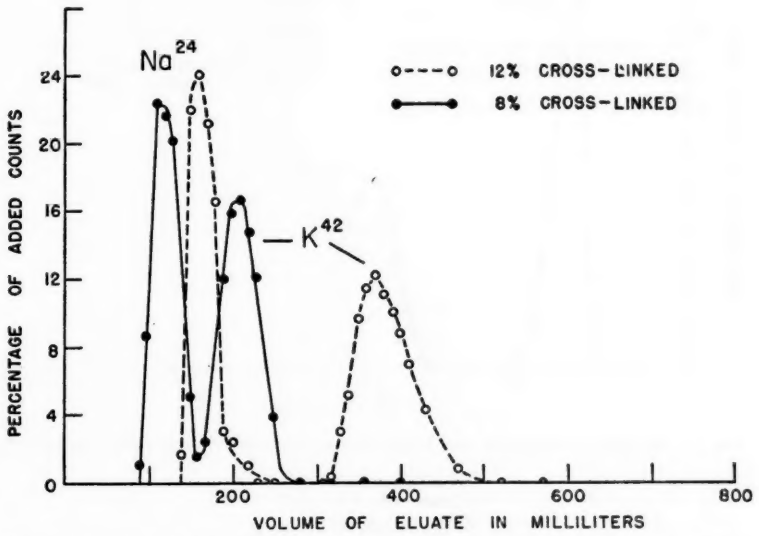

FIG. 2. EFFECT OF CROSS-LINKAGE ON ELUTION CURVES OF SODIUM AND POTASSIUM

Exchanger—Dowex 50
Column—5.5 × 1.6 cm.
Mesh—minus 325
Elutriant—0.26 N HCl
Flow rate—0.74 ml./min.
Room temperature

TABLE I

Effect of column length, elutriant concentration, and temperature on elution curves of sodium and potassium

Effect of:	Na^{24} Peak*			K^{42} Peak*		
	Position (*ml. eluate*)	Height (*% activity*)	Half-width (*ml. eluate*)	Position (*ml. eluate*)	Height (*% activity*)	Half-width (*ml. eluate*)
a. Column length						
5 cm.	130	21	40	310	12	74
5.5 cm.	140	24	38	390	10.5	84
b. Elutriant concentration						
0.13 N	170	14.5	64	500	4.5	170
0.26 N	100	24	44	280	8.5	110
c. Temperature						
20° C.	170	14.5	64	500	4.5	170
80° C.	150	17.5	46	330	6.5	120

Resin conditions:

a. 325 mesh, 12 per cent cross-linked, column diameter 1.6 cm. Elutriant is 0.26 N HCl flowing at rate of 0.74 ml./min. at room temperature.

b. 200–400 mesh, column dimensions 4.0 × 1.6 cm. Elutriant is HCl flowing at rate of 0.74 ml./min. at room temperature.

c. 200–400 mesh, column dimensions 4.0 × 1.6 cm. Elutriant is 0.13 N HCl flowing at a rate of 0.74 ml./min.

* The position of the Na^{24} and K^{42} peak is calculated in terms of ml. of eluate. The peak height is expressed as per cent of added counts found in the tube containing the highest activity. The half-width of the peak, a measure of its sharpness, indicates the volume in ml. of eluate across the peak at a point half-way to the top.

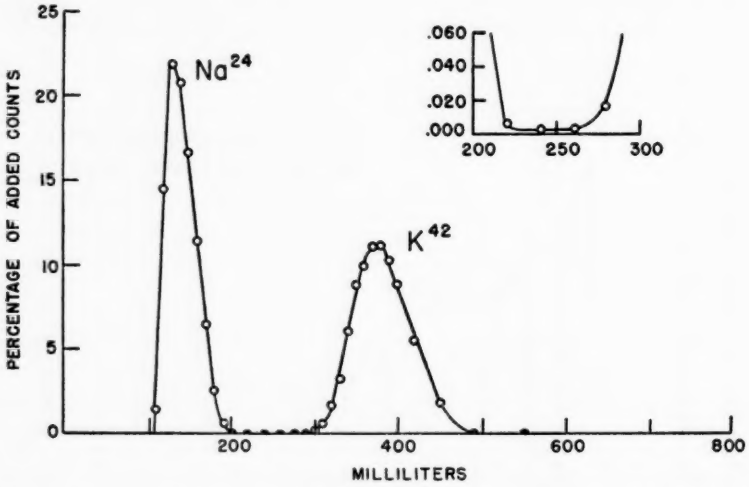

FIG. 3. OPTIMAL CONDITIONS FOR RESIN SEPARATION OF SODIUM FROM POTASSIUM

Exchanger—Dowex 50
Column—8.0 × 1.3 cm.
Mesh—minus 400
Flow rate—0.74 ml./min.
Cross-linkage—12%
Room temperature

The insert which shows a 100-fold magnification of the region between 200 and 300 milliliters indicates the negligible amount of cross-contamination in the valley between the peaks. These curves do not demonstrate the change in eluting acid normality and flow rate utilized to decrease the time for potassium elution.

the time necessary to effect the separation. An 8.0 cm. column was found to be the optimum length for our purpose.

The flow rate of eluting acid chosen was the fastest rate which would provide equilibrium conditions as judged from the symmetry of the peaks. An unimpaired flow was most easily achieved in systems which were kept constantly full of fluid to avoid the formation of air-locks. The optimal flow rate was found to be 0.74 ml. per min.

Increasing the normality of the eluting hydrochloric acid sharpened the peaks and hastened the appearance of the initial sodium peak. However, this advantage was limited by the fact that the potassium peak was moved closer to the sodium peak as the acid normality was increased. Table Ib shows the effect of changing the eluting acid from 0.13 N to 0.26 N. The most desirable acid concentration was determined to be 0.26 N hydrochloric acid.

The effect of raising the temperature provided the same advantages and limiting factors as increasing the normality of the eluting acid in that as the peaks were sharpened the K^{42} peak moved closer to the Na^{24} peak. The temperature of the resin column was increased by wrapping resistance wire around the glass column above the resin so that the entrance temperature of the eluting acid was raised to 80° C. The results of this temperature increase are shown in Table Ic. Since it was appreciably more difficult to operate at high temperatures than at room temperature this procedure was not adopted.

OPTIMUM SEPARATION CONDITIONS

Because of the numerous variables involved, approximately one hundred sets of elution curves were determined before the final conditions were chosen. The conditions for separation of sodium from potassium found to be optimal for our purposes were: a column of Dowex 50 resin of minus 400 mesh, 12 per cent cross-linked, 8.0 cm. in length and 1.3 cm. in diameter. The desirable eluting acid was 0.26 N hydrochloric acid with a flow rate of 0.74 ml. per min. Figure 3 shows the separation achieved under our optimum conditions; the insert indicates the negligible amount of cross-contamination between the two alkali ions. Be-

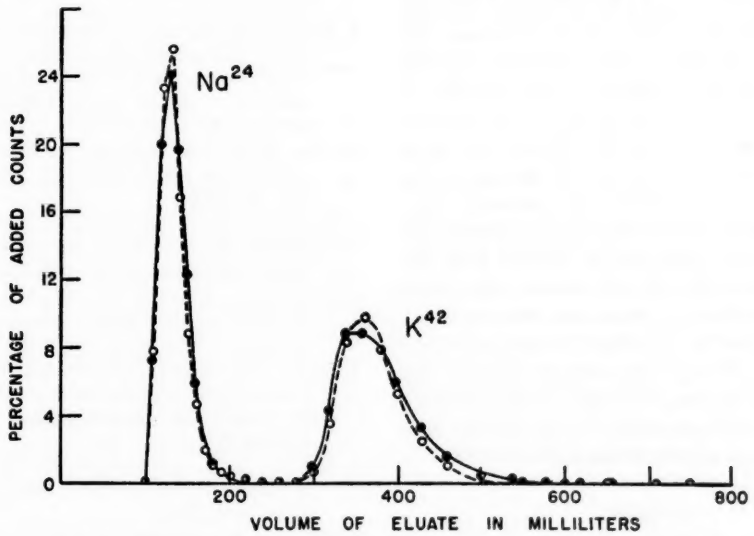

FIG. 4. REPRODUCIBILITY OF ELUTION CURVES OF SODIUM AND POTASSIUM
Exchanger—Dowex 50
Column—5.0 × 1.6 cm.
Mesh—minus 325
Flow rate—0.74 ml./min.
Cross-linkage—12%

cause of the character of the individual curves which rise sharply from the baseline but tail off as they return, sodium may appear in the potassium fraction, whereas the reverse does not occur. The magnitude of the sodium contamination of the potassium fraction as determined by radioactivity measurements of Na^{24} in the potassium eluate is of the order of 0.008 per cent of the initial amount of sodium placed on the column, well within the permissible cross-contamination.

The excellent reproducibility of the elution curves in different columns with the same batch of resin is shown in Figure 4. Integration of the areas under the curves indicate that 96 to 100 per cent of the added activity is recovered in the eluate; these data represent complete recovery to the accuracy of these determinations.

The time necessary for the sodium fraction to be eluted from the column after the sodium and potassium have been added is approximately 3.5 hours. The first 80 ml. of eluate, including approximately 40 ml. of the supernatant fluid originally added to the column, is discarded since it contains no sodium or potassium. The sodium fraction is then collected in the following 120 ml. Another 60 ml. of eluting acid is then run through the column and discarded since it represents the valley between the peaks. The potassium fraction may then be recovered quickly in the next 80 ml. of effluent by doubling the speed of flow and increasing the normality of the eluting acid from 0.26 N to 2.6 N. The total time for the separation and collection of the sodium and potassium fractions under these conditions is approximately six hours. Once the separation of sodium from potassium has been effected, the sodium and potassium eluate fractions are evaporated down to 10 ml. and each analyzed for electrolyte concentration and radioactivity. These measurements provide the necessary data for the calculation of exchangeable body sodium and potassium by the isotope dilution technique, as will be shown in Part II (2).

SUMMARY

A relatively rapid and efficient method for the separation of sodium and potassium in serum by ion-exchange chromatography has been presented. Various factors affecting the separation of these alkali metals by this technique have been discussed and illustrative experimental data have been presented. The resin separation of sodium and potassium is to be utilized in the simultaneous measurement of exchangeable body sodium and potassium.

ACKNOWLEDGMENTS

We should like to express our thanks to Mr. Bradford D. Pearson for his assistance in making the radioactive analyses. This work has been supported in part by the Atomic Energy Commission and in part by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-135.

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II. THE SIMULTANEOUS MEASUREMENT OF EXCHANGEABLE BODY SODIUM AND POTASSIUM UTILIZING ION EXCHANGE CHROMATOGRAPHY

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A technique based on the isotope dilution principle for the measurement of exchangeable body sodium in man has been devised and evaluated by Forbes and Perley (1) and Miller and Wilson (2), and a similar technique for exchangeable body potassium has been developed by Corsa, Olney, Steenburg, Ball, and Moore (3). Once the individual techniques for the measurement of exchangeable body sodium and potassium had been devised, the desirability of carrying out these measurements simultaneously became apparent because of the important biochemical inter-relationships that have been pointed out between these two alkali metals (4–6). Since the metabolic state of a patient may vary considerably during even a relatively short period, single exchangeable body sodium and potassium measurements carried out several days apart are not a satisfactory substitute for simultaneous measurements.

Such simultaneous measurements, based on the isotope dilution principle, require at present the separation of sodium from potassium prior to the radioactivity determinations. James, Brooks, Edelman, Olney, and Moore (7) have described a technique for these measurements in which classical chemical methods are used to separate the electrolytes. In the present study we have made use of ion exchange chromatography (8) for the separation. At the outset, our primary concern has been with the reproducibility of this technique for the simultaneous measurements of exchangeable body sodium and potassium in normal young adults.

"Exchangeability" of sodium and potassium

Twenty-four hours has been chosen by previous investigators (1–3, 7) as a satisfactory and convenient equilibration period. Estimated values in man indicate that approximately 82 per cent (1) of the total body sodium and 90 to 95 per cent of the total body potassium (3) are exchangeable in twenty-four hours. These measurements must be considered as preliminary since their accuracy depends on an exact knowledge of rates of electrolyte exchange in all areas of the body, data which are not yet completely available in man. In the case of sodium, bone and brain constitute areas of slow exchange (1, 2, 9), while in the case of potassium, red cell, brain, and bone exchange less rapidly than do other regions of the body (3, 10). Even though the measurement of exchangeable sodium and potassium of the body does not correspond to the total body content of these elements, it does furnish an index of total body sodium and potassium which probably varies directly with total body electrolyte content.

EXPERIMENTAL METHOD

a) *General outline*

Known amounts of radioactive sodium (Na^{24}) and of radioactive potassium (K^{42}) are injected intravenously. After a twenty-four hour equilibration period, a blood sample is drawn and sodium and potassium radioactivity and concentration (*i.e.*, specific activity) of the protein-free serum supernatant are determined. We have described in detail in Part I (8) the use of a sulfonic acid cation exchange resin, Dowex 50, to effect the separation of Na^{24} and K^{42} in order to measure their radioactivity separately as is required for these determinations by our method. Once the sodium and potassium fractions are separated, their respective specific activities $\left(\frac{\text{counts/min.}}{\text{mEq.}}\right)$ are determined. The exchangeable body sodium and potassium are then calculated from these data and from the Na^{24} and K^{42} activities in a 24-hour urine sample according to the following standard isotope dilution equation:

$$(1) \qquad E = \frac{C_i - C_s}{[C_i]/[E]}$$

in which E = amount of exchangeable body electrolyte in mEq.

C_i = total amount of injected radioisotope in counts per min.

C_e = total amount of excreted radioisotope (24-hour period) in counts per min.

$[C_f]$ = concentration of radioisotope found in the body fluid (serum in our case) in counts per min. per liter.

[E] = concentration of naturally occurring electrolyte in body fluid in mEq. per L. $[C_f]/[E]$ = specific activity.

b) *Preparation of radioactive material*

Na^{24} and K^{42} were obtained from the Atomic Energy Commission at the Brookhaven National Laboratory in the form of the carbonate, converted to the corresponding chloride salt by the addition of 1 N hydrochloric acid, and the solutions adjusted to a pH of 7. These salts were in turn diluted in distilled water to a sodium concentration of approximately 15 mEq. per L. and to a potassium concentration of about 155 mEq. per L. and assayed for activity against a uranium standard. The radioactive solutions were then further diluted in isotonic saline for injection purposes so that the 50 ml. injected would contain approximately 125 μc. of Na^{24} and 250 μc. of K^{42}. The cation concentrations of the final solutions for injection were approximately 155 and 3 mEq. per L. of sodium and potassium, respectively. These solutions were then neutralized with 0.1 N NaOH to a pH of 7.4 and sterilized in an autoclave.

c) *Preparation of resin*

The Dowex 50 resin, 12 per cent cross-linked, minus 400 mesh, and in the hydrogen cycle was obtained from Dow Chemical Company.[1] Resin columns were made up in glass tubing 1.3 cm. in diameter and rested on sintered glass filters of medium porosity. The resin was suspended in water, poured into the glass column until the particles had settled to a height of 8 cm. and then capped with glass wool. To insure complete freedom of the resin from traces of sodium and potassium all resin columns were washed before use with 300 ml. of 3 N hydrochloric acid followed by distilled water until the eluate was neutral to litmus. By means of this procedure the resin could be reused an indefinite number of times, care being taken to resuspend the resin and repack the column before each separation.

d) *Measurement of radioactivity and electrolyte contration*

The radioactivity was measured by use of a dip-counter described by Solomon and Estes (11) which had ben placed in a special stand designed by Dr. C. V. Robinson. This counter had a glass window thickness of about 30 mg. per cm^2. and could accommodate 8 ml. samples. The coefficient of variation of sets of 12 identical samples counted with this equipment was 2 per cent. The sodium eluate fraction was counted in duplicate, each sample for 10,000 counts, requiring about seven minutes apiece. Because of the low concentration of serum potassium, the potassium fraction was counted to 5000 counts requiring about 20 minutes. Potassium samples were counted in duplicate whenever possible. Duplicate standard solutions of Na^{24} (dilution 5:1000) and K^{42} (dilution 1:1000) were made up from the solutions prepared for injection. These were each counted in triplicate to 10,000 counts each. An end-window Geiger tube (2.5 mg. per cm^2. window) was used for urine counting. All data were corrected for background counts, dead time loss when necessary, and for radioactive decay.

Sodium and potassium concentrations in the eluate fractions were determined in duplicate on a Baird Associate Flame Photometer utilizing an internal standard. To avoid sodium and potassium contamination, all glassware and tubing was rinsed before use with dilute hydrochloric acid and distilled water. Sodium and potassium recovery experiments showed that the error of each flame determination was of the order of 1.5 per cent.

e) *Injection, and sodium and potassium separation*

Fifty ml. syringes for injection of radioactivity were calibrated by weighing the amount of water they would deliver and making the appropriate temperature correction. All injections were of 50 ml. volume to minimize the error of injection which was found to be 0.3 per cent. They were administered intravenously *via* the tubing of a 5 per cent dextrose in water infusion. Intravenous injection through the tubing of an infusion was found to be less subject to mishap than direct intravenous injection of volumes as large as 50 ml. Approximately 125 μc. of Na^{24} and 250 μc. of K^{42} were injected into the subject from separate syringes.

The whole body radiation resulting from this radioactivity computed by the method of Marinelli, Quimby, and Hine (12), is 0.45 equivalent roentgens. Since this dosage was used twice or, rarely, three times, several weeks apart, the total body radiation administered was considered within tolerance limits.

After a twenty-four hour equilibration period, 50 ml. of blood was drawn from the subject following an overnight fast. All urine passed during this 24-hour period was also collected. After the 24-hour blood sample was drawn, the serum was separated and the serum proteins precipitated with 15 ml. of 10 per cent trichloracetic acid (C.P.). The precipitate was then spun down at 1280 G for 10 minutes and the clear supernatant added to the resin column at a rate of 1.5 ml. per minute. The precipitate was then washed with 10 ml. of 10 per cent trichloracetic acid; after centrifugation, this supernatant was also added to the resin column. Twenty-six hundredths N hydrochloric acid (C.P.) was then passed through the column at a rate of 0.74 ml. per min. The elutriant was drawn through the column by a pump

[1] We should like to express our thanks to the Dow Chemical Company for their kindness in supplying resin and their advice in its use.

described in Part I (8) which insured a uniform rate of flow through the system. The pump could regulate the flow through four columns in parallel, thus making possible four simultaneous separations.

After the initial 80 ml. of eluate had been discarded because it contained no electrolyte, the remainder of the eluate was collected in three fractions by volume measurement.[2] The first 120 ml. fraction contained the sodium of the serum, the next 60 ml. made up the valley between the peaks, while the last 80 ml. contained the potassium fraction. In order to collect the potassium fraction as quickly as possible, we routinely increased the normality of the eluting acid to 2.6 N and doubled the rate of flow before eluting the last 80 ml. Under these conditions, the total time necessary for collection of all samples was approximately six hours. The data given in Part I (8) support the choice of these volume relationships and show that the curves are regularly reproducible.

Each of the three fractions was then evaporated down to a few ml., made up to 10 ml. with distilled water and mixed thoroughly. Eight ml. were then transferred to the dip-counter for radioactivity measurements. After counting, the same samples were analyzed for sodium and potassium concentration by flame photometry. Initially, sodium and potassium concentrations were determined on all three fractions. No determinations, however, other than the sodium analysis of the sodium fraction and the potassium analysis of the potassium fraction are necessary for the calculation of exchangeable body sodium and potassium, respectively; the other concentration measurements serve merely as checks on possible cross-contamination of the samples. Since we have never found measurable potassium in the sodium fraction, this measurement, along with flame analysis of the valley fraction, can be abandoned. Analysis of the potassium fraction for sodium is the only determination for cross-contamination that need be carried out.

f) *Measurement of excreted radioactivity*

Since the 24-hour urine sample usually contains only about 5 per cent of the injected dose of Na^{24} (1) and K^{42} (3), it was not necessary to use the resin column to make an accurate measurement of these radioisotopes in the urine. For the urine measurements we have made use of a differential absorption technique with an aluminum absorber as suggested by others (7). Since the beta rays of K^{42} are of higher energy than those of Na^{24},[3] the 166 mg. per cm^2. aluminum absorber we utilized transmits 72 per cent of the radiation from K^{42} and only 21 per cent of the radiation from Na^{24}. Urine samples were made up in triplicate, by evaporating 1 ml. of urine in aluminum planchets under an infra red lamp and were counted under an end-window Geiger tube with and without the 166 mg. per cm^2. absorber in place and with constant geometry. The counting rate of the Na^{24} and K^{42} in the urine samples could be obtained by solving the following simultaneous equations:

$$S = Na + K \tag{2}$$

$$s = 0.21\ Na + 0.72\ K \tag{3}$$

where S = counts per min. of planchets without absorber; s = counts per min. of planchets with absorber; Na = counts of radioactive sodium; and K = counts of radioactive potassium.

To calculate the total 24-hour urine activity, it is necessary to correct the end-window counts to dip-tube counts; this is accomplished by measuring the relative efficiencies of the two counters for Na^{24} and K^{42}. The over-all error in the urine method is between 3 and 5 per cent which is less than 0.3 per cent of the injected dose under all conditions of urine electrolyte composition.

The amounts of sodium and potassium lost in feces and through the skin (2, 13–16) are so small (totalling about 0.5 per cent of the injected dose) as not to have any significant effect on the body electrolyte measurements under most conditions. Under extreme conditions of diarrhea or sweating, however, it is possible that significant amounts of radioactivity could be lost *via* these routes. We have made no attempt to correct for this small electrolyte loss.

RESULTS AND DISCUSSION

Reproducibility of the method

In order to determine the reproducibility of the method, exchangeable body sodium and potassium measurements were repeated in the same normal young adult subjects at intervals of one to two weeks and in two instances several months apart. In a single subject, simultaneous measurements were made on three occasions. Thirteen simultaneous body sodium and potassium measurements were carried out in six patients. The data are presented in Table I. It is assumed that these normal subjects on normal diets and activities were in electrolyte balance during the experimental period.

The difference between pairs of exchangeable body electrolyte measurements are expressed as the difference of these measurements from their means in per cent of the mean. In the case of sodium, the differences vary from 0.7 to 3.8 per cent and in the case of potassium the differences range from 0.3 to 2.0 per cent. Coefficients of variation for a single determination determined from these

[2] A simple modification of a two-pan laboratory balance made it possible to collect each fraction automatically. The collection flask was placed on one pan of the balance, and the tares and weights adjusted so that the balance would come to equilibrium when the desired volume had been collected. A mercury switch which controlled the mechanical pump was attached to the cross-beam of the balance, so that the circuit to the pump was broken as soon as the balance came to equilibrium.

[3] See footnote 1 in Part I (8).

TABLE I

Reproducibility of simultaneous exchangeable body sodium and potassium measurements

Subject	Date	Sex	Age (yrs.)	Wt. (Kg.)	Exchangeable body Na (mEq.)	Differences from mean as % of mean	Exchangeable body K (mEq.)	Differences from mean as % of mean
R. K.	5/8/53	M	29	66.6	2,978	±1.0	3,424	±0.9
	5/15/53			67.3	2,917		3,487	
D. F.	3/19/53	M	29	68.2	3,022	+3.8	3,539	
	3/26/53			68.3	2,853	−1.9	—	±1.5
	5/7/53			68.2	2,854	−1.9	3,430	
F. M.	4/8/53	M	29	73.6	3,150		3,612	
	6/10/53			73.4	3,105	±0.7	3,705	±1.3
E. G.	4/1/53	F	21	48.5	1,875		2,161	
	4/15/53			47.6	1,986	±2.9	2,148	±0.3
M. H.	4/8/53	F	21	51.8	2,139		2,365	
	4/15/53			52.2	2,073	±1.5	2,274	±2.0
C. W.	5/8/53	F	22	50.6	2,102		2,007	
	5/15/53			50.9	2,068	±0.8	2,078	±1.8
					Coefficient of Variation	±2.6%		±2.0%

data are ± 2.6 per cent and ± 2.0 per cent for the exchangeable body sodium and potassium measurements, respectively, indicating a high degree of reproducibility. The predicted standard deviation of the method as determined from the analytic sum of the measured errors of injection, flame analyses, and radioactivity measurements is ± 2.5 per cent of the exchangeable electrolyte value, agreeing well with the reproducibility observed. These figures are in close agreement with those obtained by Corsa, Olney, Steenburg, Ball and Moore (3) in the single exchangeable body potassium measurement technique. These investigators have estimated that a difference of ± 4 per cent between determinations is significant (approximately two times the coefficients of variation obtained by us for a single determination). It should be pointed out, however, that the coefficient of variation for a difference between determinations is appreciably greater than the coefficient of variation for any single measurement, as given in Table I. In the present case the coefficients of variations for differences are 3.7 per cent for sodium and 2.8 per cent for potassium.[4] Consequently, we consider that differences of ± 7.5 per cent for sodium and ± 5.5 per cent for potassium (approximately two times the coefficient of variation for the differences) represent conservative valid criteria for significant differences between measurements.

[4] Coefficient of variation (for differences) = $\sqrt{(CV)^2 + (CV)^2}$ where CV = coefficient of variation for a single reading.

Urinary excretion

In our normal subjects the 24-hour excretion of Na^{24} varies from 3.3 to 11.8 per cent of the injected dose with a mean of 6.5 per cent as is shown in Table II. The 24-hour excretion of K^{42} varies from 2.1 to 7.2 per cent with a mean of 3.6 per cent. These values are of the same magnitude as those reported previously by others (1–3, 17). As these investigators have also noted, the amount of Na^{24} and K^{42} excreted in the urine varies directly with the total amount of sodium and potassium excreted, as is suggested by the data in Table II where mean excretion values for natural sodium

TABLE II

Twenty-four hour excretion of radioactive and natural sodium and potassium *

	% Na^{24} excreted	% body Na excreted	% K^{42} excreted	% body K excreted
Mean	6.5	5.7	3.6	2.5
Range	3.3–11.8	2.0–9.0	2.1–7.2	1.5–4.2

* Na^{24} and K^{42} excretions are expressed as percentage of administered dose while stable Na and K in the urine are expressed as percentage of the corresponding exchangeable body electrolyte value. These urinary data are derived from sixteen combined measurements in eleven patients.

TABLE III

Exchangeable body sodium and potassium of normal young adults

Subject	Sex	Age (*yrs.*)	Height (*cm.*)	Weight (*Kg.*)	Na_e (*mEq.*)	Na_e (*mEq./Kg.*)	K_e (*mEq.*)	K_e (*mEq./Kg.*)	Na_e/K_e
D. F.	M	29	180	68.2	3,022	44.3	3,539	51.9	0.85
A. K.	M	27	170	68.0	2,899	42.6	3,709	54.5	0.78
F. M.	M	29	176	73.4	3,105	42.3	3,705	50.5	0.84
R. K.	M	29	170	66.6	2,978	44.7	3,424	51.4	0.87
W. G.	M	26	174	72.5	2,969	40.9	3,407	47.0	0.87
M. S.	F	26	161	51.6	2,002	38.8	1,994	38.6	1.00
E. G.	F	21	153	48.5	1,875	38.7	2,161	45.0	0.87
M. H.	F	21	165	51.8	2,139	41.3	2,365	45.4	0.90
M. B.	F	25	159	59.2	1,999	33.8	2,103	35.5	0.95
C. W.	F	22	165	50.6	2,102	41.5	2,007	39.7	1.10
V. D.	F	23	163	58.5	2,127	36.4	2,663	45.5	0.80
C. J.	F	27	169	54.9	2,626	47.8	2,629	47.9	1.00

and potassium are expressed as a percentage of the exchangeable body sodium and potassium for comparison with the Na^{24} and K^{42} excretion data. These excretion data indicate a relatively rapid equilibration of these radioisotopes with their miscible body pools so that excretion of both radioactive and stable isotopes parallel each other.

Normal values

The data obtained in our group of normal young adults between the ages of 20 and 30 years may be used for comparison with those of other investigators. The subjects, chiefly nurses and doctors, were on their usual diets and activities during the experimental period. The values obtained by us are presented in Table III, and are expressed in terms of body weight (mEq. per Kg.) in agreement with the practice of previous investigators (1–3, 17). However, when body sodium and potassium are expressed in this fashion wide ranges of normal values are found even within the confines of a relatively homogeneous group of the same sex and age, because of individual variations in the body content of fat (electrolyte poor) and muscle (potassium rich). Nonetheless it can be seen from Table IV that our values in a limited series of exchangeable sodium measurements (mean values 43.9 mEq. per Kg. for males and 39.7 mEq. per Kg. for females) and exchangeable potassium measurements (mean values 51.1 mEq. per Kg. for males and 42.4 mEq. per Kg. for females) agree well with those of other investigators,

TABLE IV

Exchangeable body sodium and potassium in normal subjects

Investigator	Exchangeable body sodium* (*mEq./Kg.*)		Exchangeable body potassium* (*mEq./Kg.*)	
	Males	Females	Males	Females
Corsa, Olney, Steenberg, Ball, and Moore (3)			46.3† (37.0–57.1)	
Forbes and Perley (1)	41.9 (32.3–54.1)	39.5 (35.7–41.6)		
Aikawa, Harrell, and Eisenberg (17)				31.5 (25.1–35.9)
Miller and Wilson (2)	43.7 (39.5–47.9)	42.3 (39.4–44.8)		
Moore, Edelman, Olney, James, Brooks, and Wilson (18)	42.1 (36.1–48.3)	39.6 (34.4–45.7)	46.3 (35.6–53.6)	39.0 (28.0–47.2)
Arons, Vanderlinde, and Solomon	42.9 (40.9–44.7)	39.7 (33.8–47.8)	51.1 (47.0–54.5)	42.4 (35.5–47.9)

* Data expressed as mean values with the range indicated in parentheses.
† These data are also included in the later, more detailed report of Moore *et al.* listed below.

except for Aikawa, Harrell, and Eisenberg (17) whose mean value for exchangeable potassium in young adult females is considerably and unaccountably lower.

The wide range of normal data for exchangeable body sodium and potassium points out the difficulty of establishing any single value in a diseased individual as being definitely abnormal for that individual. At present, the most reliable information to be derived from exchangeable body electrolyte measurements is in patients at the beginning and end of periods of observation (such as therapeutic periods) so that *changes* in body composition may be interpreted rather than isolated measurements.

Indeed, in the absence of serial measurements, it may well be that the ratio of the exchangeable body sodium to exchangeable body potassium will provide more significant information in the individual patient at a given time than will isolated exchangeable electrolyte values. Thus, the mean Na_e/K_e ratios calculated from the data in Table III are 0.84 ± 0.04 for young adult males and 0.95 ± 0.11 for young adult females. This difference, if real, may be explained by the fact that males with their greater muscle mass contain relatively more exchangeable body potassium than sodium. In this respect it is interesting to note that a virilized young adult female we studied with an adrenal tumor had a "male" Na_e/K_e ratio of 0.74 while a debilitated male with polyarteritis nodosa had an Na_e/K_e ratio of 1.7. Further studies of body sodium to potassium ratios in disease states may provide information concerning the relationship of these two ions in the body that would not be apparent from separate consideration of the exchangeable body sodium and potassium.

SUMMARY

A simple and efficient method has been presented for the simultaneous measurement of exchangeable body sodium and potassium utilizing a cation exchange resin for the separation of radioactive sodium and potassium in the serum in combination with the isotope dilution technique. Paired measurements made in normal subjects at intervals of one to two weeks and longer have indicated that the method presented gives reproducible results. Exchangeable body sodium and potassium measurements obtained in normal young adult subjects correspond well with data acquired by other investigators. Because of the wide range of normal values, it is suggested that body electrolyte measurements will yield the most accurate information when used in a serial fashion to study patients and that the ratio of exchangeable body sodium to exchangeable body potassium may provide a valuable index of body electrolyte composition.

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THE MODE OF ACTION OF A MERCURIAL DIURETIC IN MAN

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Govaerts (1) and Bartram (2) established that the diuretic action of organic mercury compounds is renal rather than extrarenal. The use of clearance techniques has further shown that the glomerular filtration rate does not increase in man during a mercurial diuresis (3, 4). The increased rate of water excretion must, therefore, be due to diminished tubular reabsorption.

Attempts to carry the analysis still further have led to divergent views. Duggan and Pitts (5) conclude that the site of action is in the distal segment of the tubule, whereas Mudge, Foulks, and Gilman (6) and Weston, Grossman, and Leiter (7) believe it is in the proximal segment. In this paper we present data which we consider to support the former view.

METHODS

The subjects were normal male medical students or doctors. Except where it is stated to the contrary, the subjects came to the laboratory in the morning, having taken nothing by mouth since the previous evening. The experiments were begun as near 9:30 a.m. as possible. Urine was collected at 30-minute intervals by voluntary micturition, without a catheter; the collection periods were usually reduced to 15 minutes at the peak of the diuresis.

The mercurial diuretic used was Mersalyl, obtained as a solid from British Drug Houses Ltd.; unless otherwise stated, the dose was 0.2 Gm., which was dissolved in 20 ml. sterile distilled water and injected intravenously as soon as possible after solution. The injection was given slowly over a period of 10 minutes to avoid possible toxic effects on the heart resulting from a momentarily excessive blood level of the drug. No theophylline was given with these injections.

During the experiments the subjects sat in a chair or reclined on a couch; their activity was restricted to that necessary for passing urine. They took nothing by mouth, except as stated in the text. Smoking was prohibited. The chemical methods used were as follows: Sodium: direct reading flame photometer; Chloride: electrometric titration (8); Inulin: Harrison (9).

[1] Ernest Hart Memorial Scholar.

RESULTS

The effect of Mersalyl in subjects on a normal diet

Figure 1 illustrates a typical experiment. After three preliminary thirty-minute periods, 0.2 Gm. Mersalyl was injected intravenously, and it can be seen that the urine sodium and chloride concentrations, initially at a level of some 300 mEq. per liter, fell sharply to about 150 mEq. per liter at the height of the diuresis. Table I shows the results of 12 such experiments. In nine of these, the effect of Mersalyl was to cause a fall in the urinary concentration of both sodium and chloride at the peak of diuresis, producing a pattern of excretion similar to that shown in Figure 1. In the remaining three experiments (R. E. W., J. E. G., and E. R. D.) Mersalyl produced the opposite effect. The diureses in the experiments on R. E. W. and J. E. G. were irregular and unsatisfactory, reaching peak rates of only 3.7 and 5.0 ml. per min., respectively, and it seems possible that some interfering factor was masking the usual response. E. R. D. had a satisfactory diuresis, but his initial urine sodium concentration was well below his plasma sodium level.

In the four experiments marked with an asterisk in Table I, the subjects drank 0.9 per cent sodium chloride solution throughout the experiment at a rate of 200 ml. per hr. The object of this was to increase the subject's reserves of sodium and chloride, in the hope that a more profuse diuresis would result when Mersalyl was given. Control experiments without Mersalyl had previously shown that drinking saline in this way caused no significant increase in urine flow even if continued for seven hours. No increase in diuresis following Mersalyl occurred, and in fact in two experiments the diuresis was unsatisfactory; in the other two, however, good diuresis with the typical concentration changes were observed. It seemed simplest, therefore, to group all four experiments

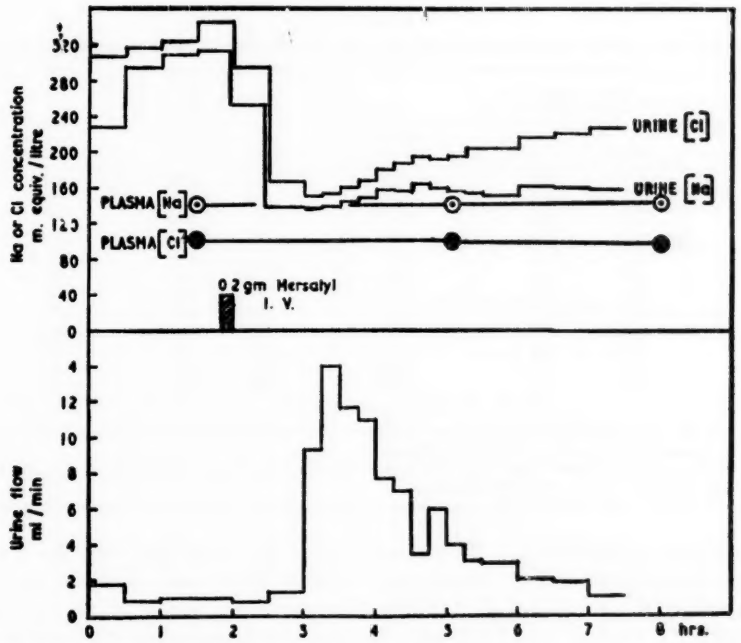

FIG. 1. THE EFFECT OF MERSALYL ON THE URINARY CONCENTRATION OF SODIUM AND CHLORIDE IN A SUBJECT TAKING A NORMAL DIET

The plasma levels of sodium and chloride are indicated by open dotted circles and closed circles, respectively.

with the rest in Table I, since the results seemed to show no obvious distinguishing features.

The effect of Mersalyl in subjects on a diet poor in salt

The marked depression of urine sodium and chloride concentrations shown in the experiments of Table I prompted us to enquire whether a similar effect would be seen if the initial urine concentrations were low rather than high. Accordingly, some normal subjects were given a diet supplying some 0.5 Gm. of sodium and 1 Gm. of chloride daily for two to three days, and then the effect of Mersalyl was observed as before. Figure 2 illustrates a typical result of this procedure. It will be seen that the injection of Mersalyl was followed by a rise rather than a fall in the urinary concentrations of sodium and chloride.

The results of seven such experiments are set out in Table II. In every case the injection of Mersalyl caused a substantial rise in the concentrations of sodium and chloride in the urine. Satisfactory diuresis was obtained in every case except one (E. R. D.), and even in this instance well marked concentration changes were seen.

It is clear, therefore, from Figures 1 and 2 and from the data contained in Tables I and II, that the effect of Mersalyl is to raise the urinary concentrations of sodium and chloride if they are low initially, and to lower them if they are high. In Figures 1 and 2 the plasma sodium and chloride levels are also indicated, by open dotted circles and closed circles, respectively. In the case of sodium the urinary concentrations at the peak of diuresis approached closely to the plasma levels in both experiments, whereas with chloride the concentrations tended, not towards the plasma level, but towards a figure some 50 per cent higher. Similar changes are found in all but three of the 19 experiments recorded in Tables I and II. This aspect of the results has been summarized in Figure 3, which indicates the changes in

$$\frac{\text{urine concentration}}{\text{plasma concentration}}$$

TABLE I

The effect of Mersalyl on urinary sodium and chloride concentrations in subjects taking a normal diet *

Subject	Urine sodium concentration (*mEq./L.*)		Plasma sodium (*mEq./L.*)	Urine chloride concentration (*mEq./L.*)		Plasma chloride (*mEq./L.*)	Urine flow at peak (*ml./min.*)
	Initial	Peak		Initial	Peak		
R. A. D.	282.5	195	152	332	193	102	8.7
R. E. W.	180	205	143.5	189	212	98	3.7
I. C. S. N.	229	163	143.5	243	160	100.5	9.9
R. B. P.	277	138	140	315	153	100	14.0
M. B. T.	190	134	146	177	134	103.5	6.8
P. S.	193	160	146	219	168	101.5	6.9
P. A. B.	166	144	143	247	150	98	12.1
E. R. D.	114	147	144	152	160	101	9.5
B. C.†	172.5	159	144	218	157	100.5	12.9
J. S. B.†	114	90	141	142	96	104	4.9
T. C. W.†	187	177.5	142.5	234	184	101	8.6
J. E. G.†	159	171	142.5	245	196	101	5.0

* The figures in the columns headed 'initial' are the means of the concentration of sodium or chloride in the periods (not less than 3 in number) before the injection of Mersalyl.

The figures in the columns headed 'peak' are the concentrations of sodium or chloride in the specimen showing the maximum rate of water excretion in each experiment.

† Subject drank 0.9 per cent sodium chloride solution, 200 ml. per hr., throughout the experiment.

(U/P ratio) for sodium and chloride. The mean of all the sodium ratios at the peak of diuresis was 0.98, with a standard deviation of 0.25; the corresponding figure for chloride was 1.50, with a standard deviation of 0.35.

The effect of Mersalyl on a maximum water diuresis

These results shed some light on the action of Mersalyl on the kidney as a whole, but, as pointed out in the discussion, they do not allow a final decision to be taken between a proximal or distal tubular site of action. It seemed that further evidence on this point might be obtained in the following way.

Current theory (based, admittedly, on indirect evidence) supposes that the fraction of water reabsorption which is under the control of the antidiuretic hormone of the posterior pituitary is carried out in the distal tubule. This water reabsorp-

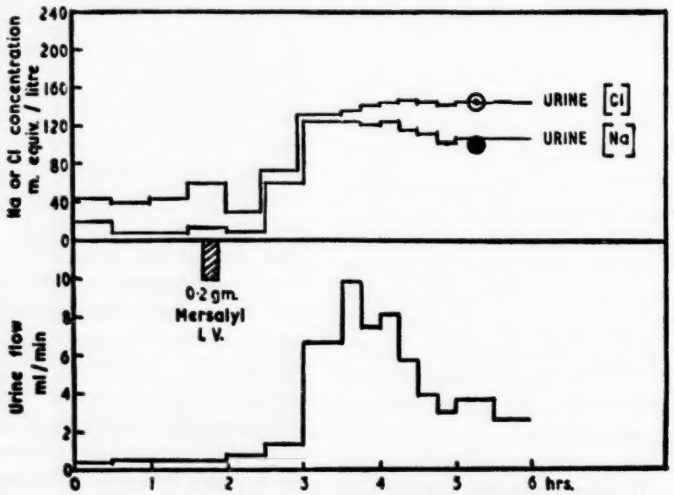

FIG. 2. THE EFFECT OF MERSALYL ON URINARY SODIUM AND CHLORIDE IN A SUBJECT TAKING A DIET POOR IN SALT

Plasma sodium and chloride indicated as in Figure 1.

TABLE II

Effect of Mersalyl on urinary sodium and chloride concentration in subjects taking a diet poor in salt *

Subject	Urine sodium concentration (*mEq./L.*)		Plasma sodium (*mEq./L.*)	Urine chloride concentration (*mEq./L.*)		Plasma chloride (*mEq./L.*)	Urine flow at peak (*ml./min.*)
	Initial	Peak		Initial	Peak		
T. C. W.	31	110	143	73	122	99.7	7.9
E. R. D.	8	134	140	41	156	100.5	3.5
I. C. S. N.	37	135	139	79	149	97	8.3
P. A. B.	12	125	144	42	136	101	9.9
B. C.	25	122	144	61	140	97	5.8
M. H.	15	126	143	31	132	94	6.2
W. R. C.	11	46	142	12	56	96.5	16.8

* The subjects took a diet supplying about 0.5 Gm. sodium and 1 Gm. chloride per day for three days prior to the experiments, except in the cases of E. R. D. and I. C. S. N., where the period was two days.

tion can be suppressed by inducing a maximum water diuresis. If Mersalyl inhibits water reabsorption in the distal tubule, then it should be incapable of producing any augmentation of a maximum water diuresis. If, on the other hand, it inhibits proximal water reabsorption, it should cause a conspicuous increase in urine flow even at the height of water diuresis.

Urine flows of the order of 15 to 18 ml. per minute can be produced without difficulty in most subjects provided enough water is drunk sufficiently rapidly; but these rates are seldom exceeded, and cannot be maintained for more than about an hour, no matter how great the intake. Moreover, the maximum rate attainable depends on the time of day, being greatest in the morning and progressively less as the day goes on (10). It was, therefore, impossible to produce a constant background of maximum water diuresis, against which the effect of an injection of Mersalyl could be presented. Instead, it was necessary to carry out two experiments in each subject: a control experiment, in which the course of the ordinary maximum water diuresis, without Mersalyl, was demonstrated, and a Mersalyl experiment, in which, at the same hour of another day, the ame amount of water was drunk and an injection of Mersalyl was given.

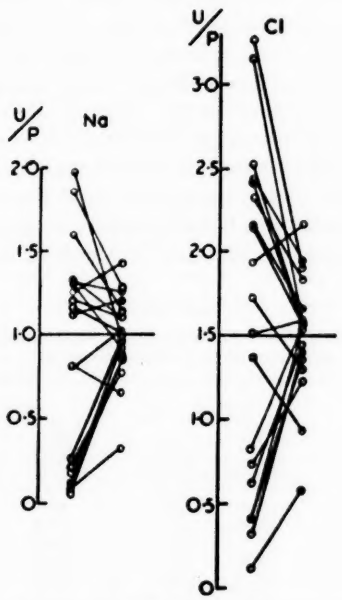

FIG. 3. CHANGES IN U/P RATIO FOR SODIUM AND CHLORIDE IN THE EXPERIMENTS OF TABLES I AND II

Each oblique line represents a single experiment: the point at its left-hand end indicates the U/P ratio before Mersalyl was given, and that at the right-hand end the ratio at the peak of diuresis.

The plan of the actual experiments is best seen from Figure 4, which shows the results obtained in subject C. M. F. At the top of the figure is indicated the water intake, and it can be seen that a peak rate of excretion of 18 ml. per min. was reached in the control experiment. In order to make the peak of the mercurial action coincide with the peak of the water diuresis, the Mersalyl was given half an hour before the first water was drunk, and in this experiment a peak rate of 18.5 ml. per min. was reached.

Table III shows the results of six such experiments. It will be seen that in every case Mersalyl produced a slight augmentation of the peak rate of urine flow; but this increase was relatively small when considered in conjunction with the rates of urine flow, and was less than 3 ml. per min. in

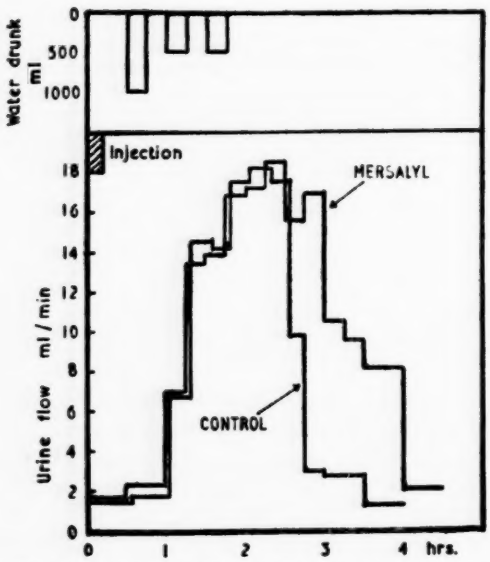

FIG. 4. DIURESIS OBTAINED (a) BY ADMINISTERING WATER ALONE (SEE UPPER PANEL) AND (b) ADMINISTERING THE SAME AMOUNT OF WATER AND INJECTING 0.2 GM. MERSALYL

The maximum rates of urine flow in the two experiments are nearly the same.

every case except one (P. A. O.). Similar results have recently been recorded by Capps, Wiggins, Axelrod, and Pitts (11). In their experiments, and in experiments by other authors using Mersalyl, depression of the glomerular filtration rate following injection of the drug was occasionally seen. Had this occurred regularly in our subjects, it might have sufficed to mask a proximal action of Mersalyl. We, therefore, repeated the experiments with simultaneous inulin clearance determinations. Figure 5 shows a typical result,

TABLE III

Effect of Mersalyl on peak rate of water excretion during maximum water diuresis

Subject	Maximum rate of urine flow (*ml./min.*)	
	Control	Mersalyl
G. M. F.	18.1	18.5
J. A. K.	17.0	19.7*
B.	9.3	10.1
E.	14.5	16.2
P. A. O.	15.1	19.2
A. W.	13.5	15.5

* Needle slipped out of vein before completion of injection. Estimated dose of Mersalyl = 0.145 Gm.

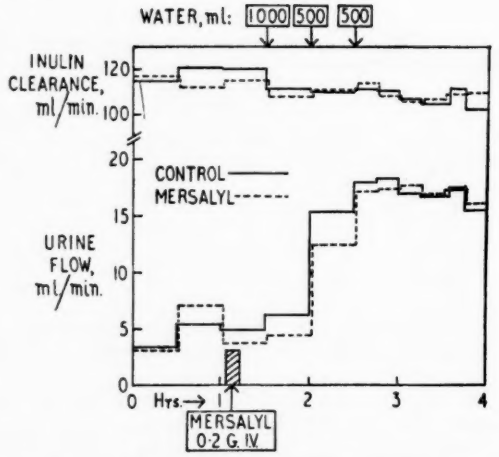

FIG. 5. URINE FLOW AND INULIN CLEARANCE IN A PAIR OF EXPERIMENTS OF THE SAME PLAN AS THOSE IN FIGURE 4

The injection of Mersalyl has no discernible effect either on the water diuresis or on the inulin clearance.

and Table IV summarizes the data from five such experiments. A conspicuous fall in filtration rate following Mersalyl administration occurred in the case of J. B., whose diuretic response was unusual in that the peak flow with water alone was greater than that with water and Mersalyl. In the remaining experiments changes in filtration were insignificant; the result in the case of W. is almost certainly an artefact due to incomplete bladder emptying. We conclude that a fall in filtration rate may sometimes follow the injection of Mersalyl, but that

TABLE IV

Changes in inulin clearance during maximum water diuresis with and without Mersalyl *

Subject	Control		Mersalyl	
	Max. urine flow (*ml./min.*)	C_{IN} (*ml./min.*)	Max. urine flow (*ml./min.*)	C_{IN} (*ml./min.*)
P. H. S.	18.3	110	17.7	106
A. L.	15.0	121	19.1	125
J. Bl.	18.0	149	13.7	117
J. Ba.	14.9	134	14.9	124
A. R. W.	16.3†	132†	13.9	113

* The figures quoted for each experiment are the maximum rate of urine flow reached, and the simultaneous inulin clearance during that period.

† Results of this period probably too high as a result of faulty bladder emptying. Figures for preceding and following periods were: urine flow, 12.0 and 14.6 ml. per min.; inulin clearance, 106 and 106 ml. per min.

this is usually so slight as not to affect the argument outlined above; on the one occasion (J. B.) when a considerable fall occurred, it was accompanied by a conspicuously reduced peak urine flow.

Effect of simultaneous administration of Mersalyl and pituitary antidiuretic hormone

Capps, Wiggins, Axelrod, and Pitts (11) studied the simultaneous effect of mercurial diuretics and pituitary antidiuretic hormone, and concluded that the latter was able to reverse the fall in urinary osmotic pressure caused by mercurials. They gave Pitressin injections after mercurials, at a time when our experience suggests that urinary sodium and chloride concentrations are likely in the natural course of events to be rising again. We have investigated this point by the opposite technique, injecting Mersalyl into subjects while they were receiving a steady infusion of Pitressin (80–120 mU per hr., with priming dose of 200 mU).

Some of these subjects were on normal and others on salt-poor diets. The results are summarized in Table V. The existence of an antagonistic effect of the Pitressin is shown by the fact that, out of four experiments with normal diet and three experiments with salt-poor diet, no diuresis was obtained in one and two experiments, respectively. In those experiments in which a diuresis was obtained, the changes in U/P ratios were comparable with those obtained in similar experiments without Pitressin (compare Figure 3). In those experiments in which there was no diuresis, the changes were towards ratios considerably higher than 1 for sodium and 1.5 for chloride, so that the average U/P for the whole series was 1.39 for sodium and 2.06 for chloride. Pitressin evidently may reduce the response to Mersalyl, and when it does so it raises the peak U/P ratios for sodium and chloride.

TABLE V

Effect of Mersalyl on urine flow and U/P ratio for sodium and chloride in subjects receiving a continuous intravenous infusion of Pitressin

Subject	Urine flow		U/P sodium		U/P chloride	
	Initial	Peak	Initial	Peak	Initial	Peak
H. J. W.	1.03	4.94	1.8	1.45	2.63	2.13
A. B.	1.21	9.66	0.65	1.11	1.62	1.58
A. L.	1.57	13.3	1.42	1.23	2.04	1.84
J. B.	1.1	1.93	1.30	1.77	2.32	2.76
D. G. L.*	0.76	9.45	0.24	0.96	0.61	1.47
N. B. P.*	0.70	0.83	0.69	1.90	0.74	2.56
G. H. G.*	0.85	1.8	0.66	1.33	0.80	2.11

* Salt-poor diet for three days preceding experiment.

DISCUSSION

The site of action of Mersalyl

Figures 1, 2, and 3 show that the effect of Mersalyl is to alter the urinary concentration of sodium in such a way that the U/P ratio tends towards unity, irrespective of whether it was initially higher or lower. Chloride behaves similarly, except that the U/P ratio tends towards 1.5.

In this connection the results obtained by Walker, Bott, Oliver, and MacDowell (12) in their micro-puncture experiments on mammalian kidneys seem to be of great interest. These workers obtained samples of fluid from the renal tubules of guinea-pigs and rats, and analyzed them for chloride, osmotic pressure and (in two instances only) sodium. The site of puncture was identified subsequently by dissection of the tubule. Owing to technical difficulties they were unable to obtain fluid from the lower part of the proximal tubule, and the observations on distal tubules are represented by only three osmotic pressure determinations. They were able, however, to obtain samples from upper proximal tubules on 49 occasions. They found that the osmotic pressure of the fluid so obtained was always close to that of the plasma, and in two instances in which the sodium concentration was determined, this also was found to be very close to that of the plasma. The chloride concentration of this fluid, however, was always higher than that of the plasma; samples taken from the middle third of the proximal tubule all showed chloride U/P ratios of about 1.4, while samples from the first third showed ratios which were lower, but tended nearer to 1.4, the lower down the tubule the puncture was made. Nothing is known as to the composition of fluid in the distal third of the proximal tubule; in the absence of evidence to the contrary it seems likely that the same ratios, 1 for sodium, 1.4 for chloride, exist there also.

The urine obtained at the peak of mercurial diuresis in our experiments in man apparently tends towards the composition of the proximal tubular fluid obtained by micro-puncture experiments in

rats and guinea-pigs, at least as regards U/P ratios for sodium and chloride, and it is tempting to suggest that this 'peak' urine may represent proximal tubular fluid which has been little if at all altered in its passage through the distal tubules. This might result either from an inhibition of distal reabsorption of salt and water as suggested by Duggan and Pitts (5) or from diminished salt reabsorption in the proximal tubule causing 'flooding' of the distal tubule by proximal tubular fluid and masking of its reabsorptive capacity, as is apparently the case in osmotic diuresis (13). The question cannot be settled by the data on U/P ratios alone, though we feel that the close approach to the ideal ratios in some of our experiments with relatively small diureses probably favors the former hypothesis. Our results, and those of Capps, Wiggins, Axelrod, and Pitts (11) show that Mersalyl in therapeutic doses does not significantly augment a maximum water diuresis. If we assume that the action of pituitary antidiuretic hormone is on the distal tubule, these results support the idea that the action of Mersalyl is similarly located. The results of administration of Mersalyl during a Pitressin infusion are too variable to allow a definite conclusion to be drawn. Thus the results reported here, while not in themselves conclusive, add to the evidence favoring the view that Mersalyl inhibits reabsorption of sodium, chloride, and water in the distal tubule.

SUMMARY

1. The injection of Mersalyl into normal human subjects caused the U/P ratio for sodium to approach 1 and that for chloride to approach 1.4, regardless of whether the initial urine concentrations were high or low.

2. Mersalyl was incapable of producing more than a slight augmentation of a maximum water diuresis.

3. When Mersalyl was given during an infusion of pituitary antidiuretic hormone, the results were variable. In some experiments, the diuresis was suppressed completely; in others a diuresis occurred, and the same changes in U/P ratio were seen as in experiments without antidiuretic hormone.

4. These results are considered to favor the view that Mersalyl inhibits reabsorption of sodium, chloride, and water in the distal tubule.

ACKNOWLEDGMENTS

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THE EFFECTS OF EATING AND OF SHAM FEEDING UPON THE ABSORPTION OF VITAMIN A PALMITATE IN MAN [1]

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It is the usual practice to test the intestinal absorption of specific nutrients in man by first feeding a test dose of the pure nutrient to the fasting subject, and then determining in samples of blood or urine taken over various subsequent periods of time the amount of the nutrient present in the blood or excreted in the urine. In the case of vitamin A absorption, such tests have been performed either to study its assimilation as a *vitamin*, or as an indicator substance correlated more or less closely with the general absorption of fats. That vitamin A in oily solution is absorbed by man in a manner closely associated with the absorption of the oil in which it is dissolved, most studies would agree (1–3). When vitamin A acetate or palmitate or natural esters are given, dissolved in oil, the normal human subject exhibits an increase in the total vitamin A concentration of his blood serum for the succeeding 3 to 8 hours. The rate at which the serum levels rise, and the maximum concentrations attained are widely variable; consequently, many arbitrary standards for "normal" vitamin A tolerance tests have been set. It is generally agreed that the normal human subject should show a rise in the serum vitamin A concentration to at least four times the fasting level, or to a level greater than 100 micrograms per 100 ml. serum, during the 4 to 6 hours following ingestion of a test dose of 75,000 to 150,000 micrograms of an esterified form of vitamin A in oily solution (2–4).

In the course of some studies with vitamin A tolerance tests, using as a test dose 100,000 micrograms of synthetic vitamin A palmitate in corn oil,[2] 5 of 16 apparently normal subjects failed to show any appreciable elevation of the serum vitamin A concentrations during the 8-hour period following the test dose. These subjects had, in addition to adequate dietary supplies of vitamin A and normal fasting serum vitamin A levels, normal serum carotene values, which in our experience almost certainly exclude the presence of defective fat absorption. Accordingly, further studies were carried out to define the nature of this apparent absorptive defect. One of the subjects became hungry during the test, and was allowed to eat a sandwich and drink some coffee. A serum sample taken immediately after this meal revealed that his vitamin A concentration had increased from 45 to 350 micrograms per cent in 20 minutes. The results of the effects of eating upon the other four normal subjects who had failed to show any absorption of the test solution when fasting were the incentive for a more systematic study.

METHODS

1. Vitamin A was determined in small quantities of serum by the spectrophotometric method of Bessey, Lowry, Brock, and Lopez (5) utilizing the principle of destruction by exposure to ultraviolet light of the vitamin A present in a xylene-kerosene extract of serum. All samples were analyzed in triplicate for both carotene and vitamin A, in a Beckman model DU spectrophotometer, at wave lengths of 460 millimicrons for carotene and 328 millimicrons for vitamin A.

2. The test material consisted of clear solutions of synthetic vitamin A palmitate in corn oil, containing approximately 10,000 micrograms vitamin A per ml. This was a very stable product, showing little or no deterioration in assayable vitamin A when kept in amber bottles in the refrigerator. A single dose of 10.5 ml. of this oil was administered by mouth or through rubber tubes as described below.

PROCEDURE

1. Of the 16 normal healthy young adults selected as controls, eight were males. The five subjects who failed to show any absorption of the test dose when administered to them fasting were given a repeat dose one week following the first test, and exactly two hours later were permitted to eat whatever they wanted, completing the

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[2] Provided through the courtesy of Hoffmann-LaRoche, Inc.

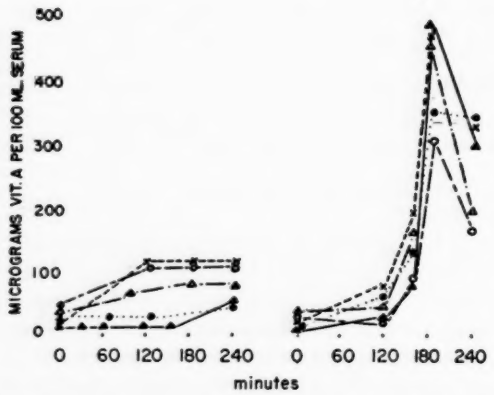

FIG. 1. ORAL VITAMIN A TOLERANCE TESTS IN FIVE NORMAL SUBJECTS

On the left are results of administering test dose to fasting subjects; on the right the same subjects ate breakfast at 120 minutes following administration of the test dose.

meal within 30 minutes. Blood samples were taken before the test dose, at two hours (immediately prior to eating), at 150 minutes, at 180 minutes, and at 240 minutes. The results may be seen in Figure 1 and Table I.

2. A second group of 16 normal control subjects and a group consisting of 32 hospitalized patients not clinically suspected of defective fat absorption were given the test dose, followed by their customary breakfast two hours later. Serum samples taken at 0, 120, 150, 180, and 240 minutes were analyzed for vitamin A and the results plotted in Figure 2.

3. In six of the normal subjects from the first group, gastric tubes were passed under fluoroscopic control into the third portion of the duodenum. A mixture of 100,000 micrograms of vitamin A palmitate and 20 ml. warm water was gently emulsified and instilled through the tube:

TABLE I

Results of oral vitamin A tolerance tests in normal subjects, using as a test dose 100,000 micrograms synthetic vitamin A palmitate in corn oil

Subject	Sex	Carotene *mcg./100 ml. serum*	Vitamin A, *micrograms/100 ml. serum*					
			Fasting	120 *min.*		150 *min.*	180 *min.*	240 *min.*
J. B.	M	132	26	56	Food ingestion	118	380	437
M. L.	F	199	46	98		162	388	401
M. M.	M	168	38	38		280	362	450
P. R.	M	132	58	200		228	300	327
L. H.	M	151	44	127		141	270	313
M. E.	F	115	44	65		211	630	705
H. W.	M	154	32	48		117	488	400
A. M.	M	138	46	97		135	288	210
M. P.	F	125	52	56		109	348	246
J. H.	M	157	46	216		248	490	248
D. H.	F	220	45	410		532	192	134
J. R.	F	116	29	218		290	554	432
L. O.	F	125	48	139		505	748	485
G. P.	M	136	50	62		332	564	542
Mean values		146	43	126		241	442	402
S.E. mean		±7.5	±2.1	±25.0		±32.5	±39.2	±35.1

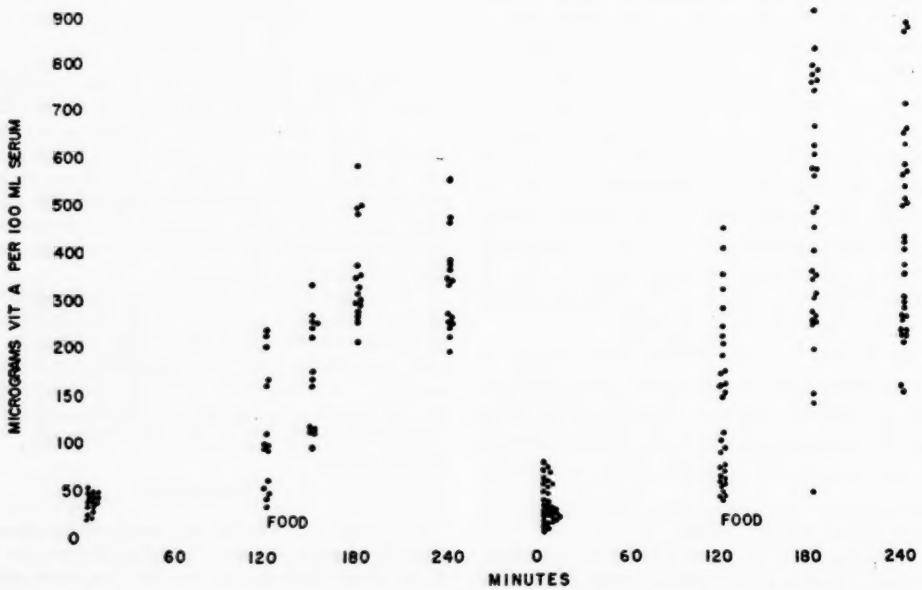

FIG. 2. ORAL VITAMIN A TOLERANCE CURVES IN AMBULATORY CONTROL SUBJECTS (LEFT), AND IN HOSPITALIZED CONTROL SUBJECTS (RIGHT)

In both groups a normal breakfast was eaten at 120 minutes following administration of test dose.

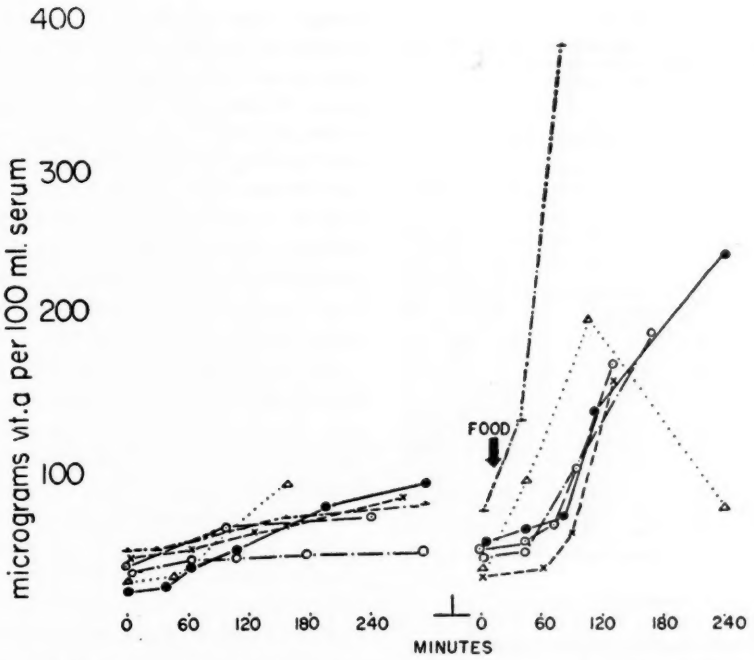

FIG. 3. INTRADUODENAL ADMINISTRATION OF VITAMIN A IN SIX NORMAL SUBJECTS

On the left are results when subjects did not eat following instillation of test dose; on the right are results when subjects ate breakfast within 30 minutes following instillation of test dose.

the tube was then withdrawn and serum samples taken over the succeeding four hours. This same group of normal subjects was then given the same test one week later, but this time the subjects were allowed to eat breakfast immediately following instillation of the test dose. There was a significant increase in the vitamin A absorption after eating, as may be seen in Figure 3.

4. In order to study the effects of an anticholinergic agent, four of these subjects then took the test dose orally, but 100 minutes later received an intramuscular injection of 1.0 mg. atropine sulfate. They ate breakfast twenty minutes following this injection, and had the usual blood samples taken. Two such tests were carried out in each subject. The results of this study are summarized in Table II.

5. In order to test the time relationships involved in the taking of food, two subjects with the intraduodenal tube in place ate breakfast, then had the test dose instilled 20 minutes later, after which the tube was withdrawn. The same two subjects one week later took the test dose first, followed within twenty minutes by the breakfast. To assess the effect of the presence of the tube itself, one of the subjects kept the tube down while eating his meal, and let it remain there during the next two hours. All five tests gave essentially the same results.

6. The same test was given to three subjects who had been totally gastrectomized within the preceding six months. One subject had an esophagoduodenostomy, two had esophago-jejunostomies; the results of all three tests were similar, and are recorded in Figure 4.

7. Sham feeding at two hours following the ingestion of the test dose was next tried; the five subjects thoroughly masticated an attractive meal, but expectorated all of it without swallowing so much as a drop of saliva; two hours later they ate, and retained, a similar meal. The results of all five tests are summarized in Table III.

8. An attempt to exclude biliary and pancreatic juice from contact with the test solution was made by "isolating" the proximal jejunum of two normal subjects between two recording balloons; the test solution of vitamin A was then instilled into this segment. In the study shown in Figure 5A, no absorption from the isolated segment could be detected, despite violent increases in amplitude of contractions and in tonus, as recorded by the two balloons; in the second part of this study, after a meal was taken, the distal balloon was deflated, allowing the vitamin A solution in the isolated loop to escape along the adjacent jejunum, and some absorption was noted. Consequently, the distal balloon was deflated when the second subject (Figure 5B) was studied, in both the fasting and the fed states.

TABLE II

Studies in normal subjects; one mg. atropine sulfate administered i.m. 100 minutes after ingestion of test dose of vitamin A palmitate

Subject		Serum vitamin A concentrations, *micrograms per 100 ml.*						
		Minutes after administration of test dose						
		0	100	120		150	180	240
M. M.	control	36		120		262	486	484
	atr. No. 1	40	atrop.	102		136	156	170
	atr. No. 2	32	atrop.	36		58	122	430
M. L.	control	38		160		222	286	400
	atr. No. 1	40	atrop.	118		130	236	220
	atr. No. 2	34	atrop.	36		44	52	182
P. R.	control	44		98		162	212	220
	atr. No. 1	40	atrop.	96		134	230	366
	atr. No. 2	48	atrop.	50		62	106	220
H. W.	control	38		42		90	250	342
	atr. No. 1	36	atrop.	60		128	140	360
	atr. No. 2	40	atrop.	64		206	228	675

Food ingestion

RESULTS

In Figure 1 are clearly seen the rapid rises in serum vitamin A concentration immediately following the taking of a meal, in five normal subjects who had failed to show any absorption when fasting. The extension of this test to a larger number of controls, as seen in Figure 2, seems to confirm the remarkable stimulatory action of the taking of food upon the level of vitamin A in the serum, although in many subjects the levels were already rising fairly rapidly prior (*i.e.*, in the serum sample taken at 120 minutes) to the taking of food. In no one of these 48 tests did the serum vitamin A concentration fail to reach either a level greater than four times the fasting concentration, or an absolute value of 100 micrograms per cent during the four hours of the test.

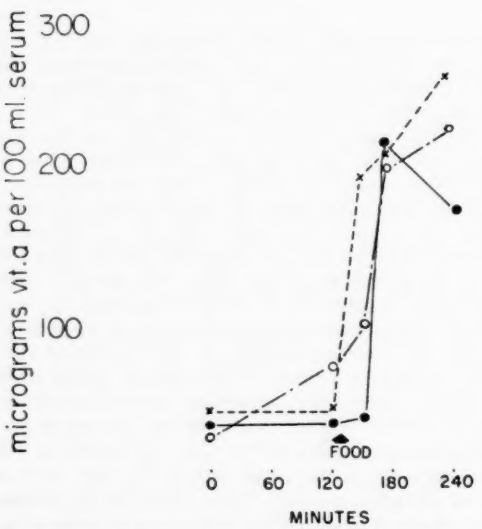

FIG. 4. ORAL VITAMIN A TOLERANCE CURVES IN THREE TOTALLY-GASTRECTOMIZED SUBJECTS

A normal meal was given 120 minutes after ingestion of the test dose.

The intubation studies depicted in Figure 3, and those described under "Procedure," paragraph 5, show that the effect of food was manifest whether the test solutions were instilled prior to eating or following the meal. That the presence of the stomach, and presumably of its acid-peptic secretions, is not requisite for this effect of food is provided by the studies on totally-gastrectomized subjects (Figure 4). More surprising are the results with sham feeding, which seems to possess stimulatory powers nearly equal to those of actual food ingestion, although the effect is not so prolonged (Table IV). The studies reported in Figure 5 were designed to prevent biliary and pancreatic juice from coming into contact with the test solution, although of course succus entericus could not be so eliminated. The administration of atropine 20 minutes prior to food ingestion had a statistically significant effect in delaying by 30 minutes the onset of the rapid rise in serum vitamin A concentration usually observed after food ingestion (Table IV). The maximum serum concentrations attained were, however, not markedly different in the control and atropinized subjects.

TABLE III

Studies with sham feeding
Serum vitamin A concentration, micrograms per 100 ml., obtained in normal subjects

Subjects	Minutes after administration of test dose								
	0	120		150	180	240		300	360
D. C.	38	64	Sham meal	84	128	225	True meal	—	255
S. D.	38	68		155	235	285		—	370
I. P.	37	83		260	—	350		400	480
J. R.	52	96		184	275	280		675	—
L. C.	60	80		206	228	256		390	620

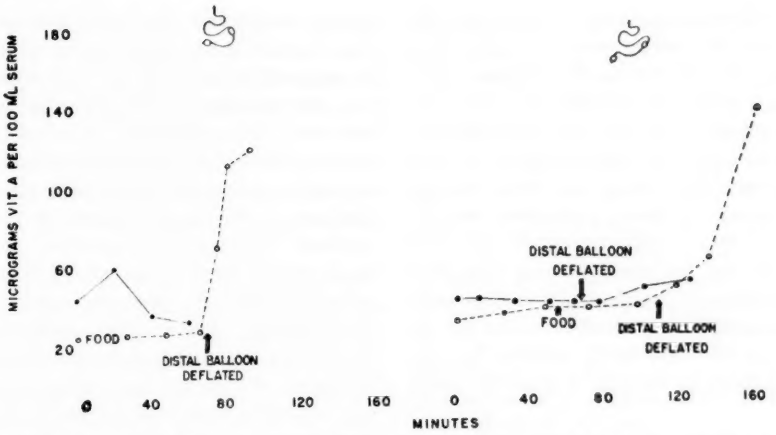

FIG. 5. A. (LEFT) STUDIES WITH TRIPLE-LUMEN TUBE "ISOLATING" JEJUNUM OF A NORMAL SUBJECT

Test solution of vitamin A instilled at 0 minutes; closed circles represent values in fasting subject, open circles in fed subject.

B. (RIGHT) STUDIES WITH TRIPLE-LUMEN TUBE "ISOLATING" JEJUNUM OF A SECOND NORMAL SUBJECT

Test solution of vitamin A instilled at 0 minutes; distal balloon deflated in each study—fasting (closed circles), and fed (open circles).

DISCUSSION

These studies are concerned with the absorption of synthetic vitamin A palmitate, a more homogeneous substance than the usual preparations of natural vitamin A esters, which contain large amounts of vitamin A acetate and variable quantities of vitamin A alcohol, both of which may be absorbed more rapidly than, or by mechanisms other than those involved in absorption of long-chain fatty acid esters. In general, the serum vitamin A levels in control subjects reported here rise less rapidly and reach lower concentrations than those reported for studies in which the natural esters have been the test solution (2–4). It would be distinctly unusual to find 5 of 16 normal subjects exhibiting "flat" tolerance tests after a test dose of natural vitamin A esters.

It seems clear from the experiments reported above that the act or thought of eating activates the mechanisms by which vitamin A enters the blood stream, so that the rate at which the vitamin A concentration rises may be markedly increased within a few minutes. The experiments seem definitely to exclude gastric emptying or secretion as necessary for this activation; the possible role of

TABLE IV

Mean rates at which serum vitamin A concentrations rise following the test dose, expressed as micrograms vitamin A per 100 ml. per minute

Type of study	No. of tests	Period A Fasting 0–120 minutes (Mean ± S. E. mean)	Period B 120–150 minutes (Mean ± S. E. mean)	Period C 150–180 minutes (Mean ± S. E. mean)
Control (Table I)	14	Fasting 0.73 ± 0.23	During food ingestion 4.1 ± 0.92	After food ingestion 7.3 ± 0.91
Sham Feeding (Table III)	5	Fasting 0.32 ± 0.11	During sham feeding 3.34 ± 0.76	After sham feeding 1.98 ± 0.46
Atropine (Table II)	8	Fasting 0.35 ± 0.19	During food ingestion 1.43 ± 0.42	After food ingestion 1.66 ± 0.46

the hormone villikinin in causing the intestinal villi to pump fat into the lacteals seems excluded here by the studies in gastrectomized subjects, since this hormone is postulated to arise from the contact of hydrochloric acid with the duodenal mucosa (6). The effects of sham feeding are reasonably clearcut; that eating and sham feeding increase the output of biliary, pancreatic, and enteric digestive juices is quite undeniable (7), but the studies with the "isolated" jejunum done here suggest that although intraluminal factors must govern the absorption of vitamin A from the intestine, they do not necessarily account for this sudden rapid entry of vitamin A into the blood stream.

The dose of atropine given in these studies produced a definite delay in the onset of the rise in serum vitamin A levels, the serum levels for the period immediately following eating being statistically lower ($P < 0.05$) than in control studies, but all four atropinized subjects did exhibit maximal concentrations in the serum within 240 minutes after taking the test dose which were comparable to those in the control studies. Such an effect resembles closely that reported by Ingelfinger, Moss, and Helm (8) in atropinized subjects fed natural vitamin A esters intraduodenally. Their studies seemed to implicate qualitative changes in digestive juices as influencing markedly the absorption of natural vitamin A esters from the intestine, but they were unable to delineate clearly the relative roles of digestive juices, intestinal motility, and other unknown factors in the absorption of vitamin A.

The rapidity of the rise in serum vitamin A which has been demonstrated in some of these cases directs attention to effects of the act or thought of eating other than those briefly discussed above. It has been known for centuries that the lacteals of animals may remain engorged with fat for hours after fed fat has disappeared from the intestine. A sudden contraction of these fat-filled lacteals would be capable of flooding the blood stream with vitamin A in a very short period of time. There are few data in the literature defining the factors which are capable of causing such sudden contractions, but it is well-established that the parenteral or oral administration of hypertonic saline has a marked stimulatory effect on lymph flow (9). Studies on the flow of thoracic duct lymph in experimental animals demonstrate marked variations in the speed and volume of this flow, but eating and drinking are usually followed by appreciable increases in both modalities (10, 11). Further studies along these lines are certainly indicated. There are many reports in the literature which make use of vitamin A test solutions either incorporated into or given with meals (3, 12). Analyses of the serum vitamin A concentrations obtained with such test solutions show them to reach higher levels more rapidly than those reported in subjects given the same test solution when fasting. The specific accelerating effect of the food or of eating is thus difficult to analyze, as compared with the technique adopted in the studies reported here, since the maximal stimulus to absorption is provided at the very beginning of the test, but few or no falsely flat tolerance tests have been described when this procedure is followed.

The clinical usefulness of the vitamin A tolerance test as described above, in which a meal is fed 120 minutes following administration of the test dose, seems well established by the results in this laboratory. In 106 tests performed on hospitalized patients 13 have failed to show a serum vitamin A level greater than four times the fasting concentration, or failed to reach 100 micrograms per cent within the four hours required by this test. All 13 of these patients have shown significant steatorrhea (*i.e.*, fecal fat representing more than 8 per cent of ingested fat) on the basis of four-day fat balance studies, and many have presented the clinical stigmata of defective fat absorption as well. It would appear that the inclusion of a meal as a stimulus is a simple and convenient way of avoiding the flat tolerance tests which may occur in normal subjects. If vitamin A has been absorbed, the act of eating should cause most of it to enter the blood stream; if it does not appear after such a stimulus, one is quite justified in assuming that it has not been absorbed.

SUMMARY

1. Normal fasting subjects may demonstrate no appreciable rise in serum vitamin A concentration during the 8-hour period following ingestion of the test dose of vitamin A palmitate in corn oil.

2. The feeding of a meal two hours after the ingestion of the test dose activates the mechanisms whereby recently absorbed vitamin A enters the blood stream. Studies done on normal and gas-

trectomized subjects, with and without eating, cast doubt that this mechanism is intraluminal. It is suggested rather that the lacteals may be activated in some way by eating to expel their contained vitamin A into the blood stream.

3. Sham feeding is almost as effective as food ingestion in activating this mechanism. Atropine seems to delay the response, but not to prevent its occurrence.

4. Failure of 13 subjects of 106 tested to demonstrate appreciable rises in serum vitamin A levels when fed a meal two hours after ingestion of the test dose of vitamin A palmitate was in all cases shown to be associated with significant steatorrhea.

ACKNOWLEDGMENT

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THE EFFECT OF MILD EXERCISE IN THE SUPINE POSITION ON THE PULMONARY ARTERIAL PRESSURE OF FIVE NORMAL HUMAN SUBJECTS [1]

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In the course of study of a series of patients with cardiopulmonary diseases of various type and degree, it was noted that the pulmonary arterial pressure rose during supine exercise in all instances. This rise was observed even in individuals with minimal pulmonary involvement and no detectable cardiac abnormality. Inasmuch as it has been stated that the pulmonary arterial pressure does not rise appreciably with mild exercise in subjects with normal cardiorespiratory systems (1–3), it seemed necessary to re-examine this question.

It is the purpose of this paper to describe in detail the pulmonary arterial pressure rise which occurs in normal man during mild, supine exercise, together with certain associated physiological changes.

METHODS

Five untrained individuals were selected for study (Table I). All were considered to be entirely healthy by the criteria of history, physical examination, electrocardiogram, and postero-anterior 6 foot chest roentgenogram.

The subjects were given .09 gram of Nembutal® orally the evening prior to catheterization. The morning of the catheterization they were again given .09 gram of Nembutal® orally, and arrived at the laboratory one hour later in the fasting state. Pulmonary artery catheterization was performed in the usual manner (4). An indwelling intra-arterial needle was introduced into the brachial artery on the opposite side. All studies were carried out with the subject in the supine position.

The resting cardiac output was then determined by collection of 6 minute expired gas samples in 100 liter Davol Douglas-Type Gas Bags and withdrawal of simultaneous blood samples from the pulmonary artery and brachial artery. The subjects were then prepared to use an apparatus designed for exercising in the supine position (5). (They had, in each instance, been familiarized with the use of the device on the day before.) Exercise stroke length was 12 inches, but actual excursion of the weight was 6 inches because of the pulley arrangement. A metronome was used to maintain rhythm. Continuous recording of the pulmonary arterial pressure-pulse contours and the pulmonary arterial mean pressure was then begun. While continuous pressure records were being made, the patient began to exercise, moving both lower extremities synchronously. After a warm-up period of 3 minutes, expired gas was collected from 3 to 4¼ minutes (designated 3.6 minutes of exercise) together with blood samples for determination of the cardiac output. Pressure recording was then resumed. At 6 minutes another collection of expired gas was begun, lasting until 7¼ minutes (designated 6.6 minutes of exercise). Exercise was then abruptly terminated. Total exercise time was 7¼ minutes in each instance. Continuous post-exercise recordings of the pulmonary arterial pressure were made for 5 minutes.

The pressure-pulse contours were recorded with a Hathaway Blood Pressure Control Unit and Oscillograph.[2] The pulmonary arterial mean pressure was detected by means of a Sanborn Electromanometer and recorded by the Hathaway Oscillograph. Recordings were calibrated with a mercury manometer after each tracing. The zero point for all pressures was 5 cm. posterior to the sternal angle of Louis with the patient supine. The pulmonary arterial mean pressure as given by the Sanborn Electromanometer was compared in each instance with the mean pressure obtained by planimetric integration of the Hathaway pressure-pulse contours and found to check within 1 or 2 mm. Hg. All pulmonary arterial mean pressures given in this paper were detected with the Sanborn Electromanometer. Systolic and diastolic pulmonary arterial pressures were obtained by averaging the values for one or more complete respiratory cycles. Measurements of all pressure tracings were rounded to the nearest integer, five-tenths having been rounded to the nearest even integer.

Blood samples were analyzed for oxygen content and capacity by the Scholander-Roughton syringe method (6). Expired gas volume was measured by means of a Precision Wet Test Gas Meter. Expired gas samples were analyzed for carbon dioxide and oxygen content [3]

[1] Aided by a grant from the Life Insurance Medical Research Fund.

[2] Type SYBP-1 Blood Pressure Recording System.

[3] Subsequent to this study it was found that the expired gas had been permitted to remain in the Davol Douglas-Type Bags too long before analysis. The oxygen uptake and cardiac output data recorded in this paper have been corrected by the application of formulas derived from

TABLE I

Five normal human subjects and their work rates

Subject	Age	Sex	Weight (*lb.*)	Height (*in.*)	Body surface area (*sq. m.*)	A-P chest diameter (*cm.*)*	Strokes per minute	Work rate (*ft-lb/min.*)
MJB	20	M	160	71	1.91	21	28	784
MM	24	M	150	70	1.83	21	30	840
MMH	22	F	103	62	1.43	18	28	784
ELM	25	F	118	65	1.58	17.5	25	700
MES	21	F	118	62	1.52	14.5	29	812
AVG							28	784

* Some investigators working in this field record "zero" pressure in the supine subject at the level 5 cm. posterior to the anterior chest wall, while others use the level 10 cm. anterior to the posterior chest wall. Inasmuch as the A-P chest diameter is usually not 15 cm., it is obvious that these levels are not exactly equivalent. Pressure differences resulting from this discrepancy may amount to as much as 5 mm. Hg.

with the Scholander micrometer gas analyzer (8). Oxygen uptake was calculated from the results of analysis of expired gas by means of the standard formula (9). All gas volumes including those for oxygen uptake and minute volume of breathing were converted to STPD (0° C., 760 mm. Hg, dry) and are given as such in this paper. The ventilation equivalents were obtained by dividing the minute volume of breathing (STPD) by the corresponding oxygen uptake (STPD). Cardiac output was calculated using the Fick principle.

experimental analysis of the rates of change of carbon dioxide and oxygen concentration in samples of gas permitted to stand in these bags (7). After derivation, these formulas were tested and it was found possible to predict initial gas concentrations from final gas concentrations within the limits of analytical error, when the elapsed time is accurately known. It is therefore felt that the oxygen uptake and cardiac output data given in this paper are quite accurate, and that the above-mentioned error in technique does not invalidate the conclusions drawn from these data.

RESULTS

Pulmonary arterial pressure

The resting pulmonary arterial systolic, mean and diastolic pressures varied from 17 to 26, 11 to 17, and 8 to 12 mm. Hg, respectively (Table II). The average resting systolic, mean, and

TABLE II

The effect of exercise on the pulmonary arterial pressure

	Subject		Rest	Exercise*								Post-exercise*					
				¼	½	¾	1	1½	2	3	5	6	¼	½	¾	1	3	5
Pulmonary arterial pressure	MJB	S	22	27	28	28	28	28	27	27	28	27	22	19	19	17	18	18
		D	12	16	16	16	17	17	16	16	16	16	12	11	8	8	9	10
		M	16	21	21	22	22	22	22	22	22	22	16	14	13	13	13	13
	MM	S	22	24	24	25	26	25	26	27	27	27	23	22	22	21	18	17
		D	8	11	12	12	12	12	12	12	12	12	8	8	9	8	7	6
		M	14	18	19	20	20	19	20	20	20	20	16	16	16	16	13	13
	MMH	S	23	28	29	31	32	32	33	33	28	28	23	23	21	19	18	18
		D	10	14	14	14	14	15	15	15	10	10	6	6	5	5	5	5
		M	15	18	19	20	20	20	21	21	17	17	13	13	12	10	10	10
	ELM	S	17	19	20	21	23	22	23	22	21	21	20	20	19	19	19	19
		D	8	10	10	11	12	11	12	12	11	11	8	7	7	7	7	7
		M	11	14	14	15	16	16	17	16	15	15	12	11	10	10	10	10
	MES	S	26	31	31	29	29	28	28	26	26	26	26	26	26	24	24	24
		D	10	14	13	12	12	12	13	14	13	13	13	12	12	9	9	9
		M	17	21	21	20	19	18	18	18	18	18	18	17	17	15	15	15
	AVG	S	22	26	26	27	28	27	27	27	26	26	23	22	21	20	19	19
		D	10	13	13	13	13	13	14	14	12	12	9	9	8	7	7	7
		M	15	18	19	19	19	19	20	19	18	18	15	14	14	13	12	12

* Time in minutes.

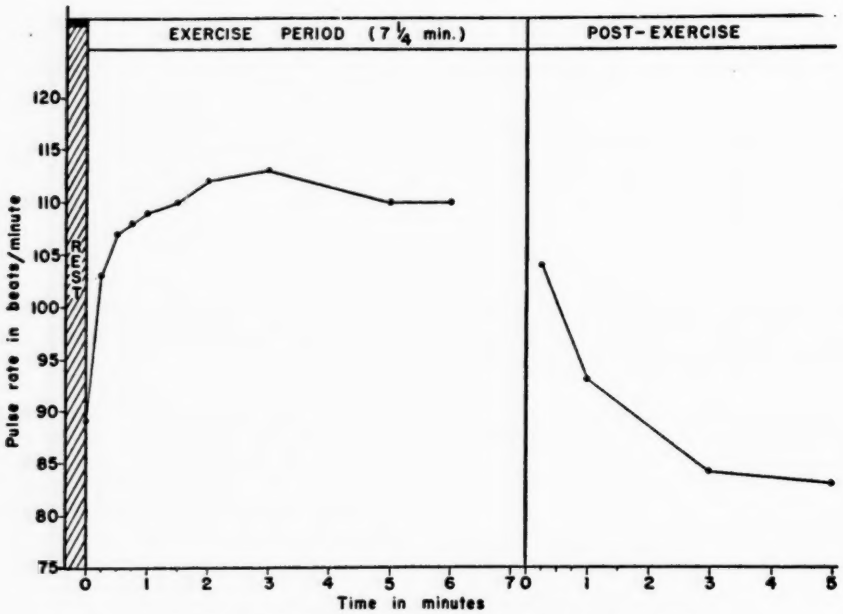

Fig. 1. EFFECT OF MILD SUPINE EXERCISE ON THE PULSE RATE. Average of 5 subjects

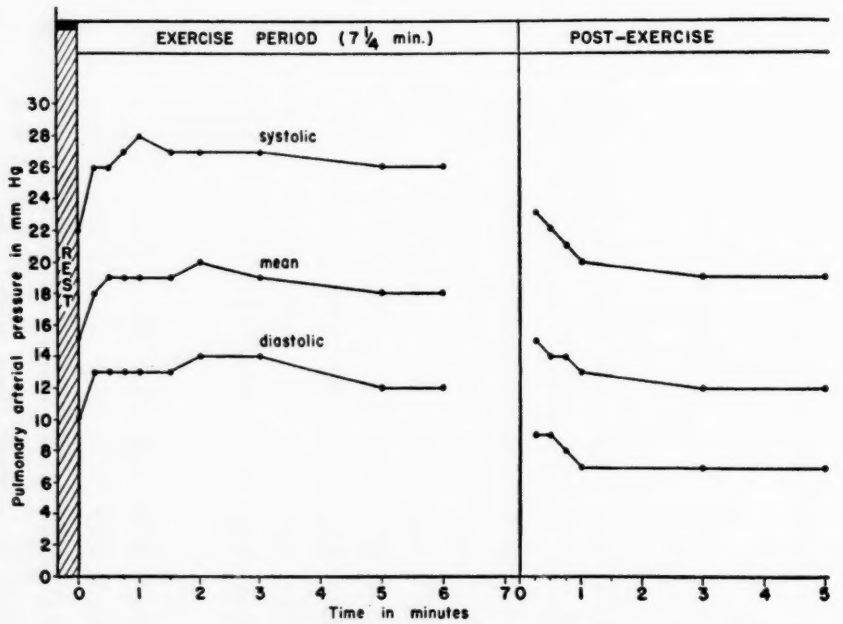

Fig. 2. EFFECT OF MILD SUPINE EXERCISE ON THE PULMONARY ARTERIAL PRESSURE. Average of 5 subjects.

TABLE III

The effect of exercise on oxygen uptake and a comparison of values obtained by use of the standard formula and by use of an average respiratory exchange ratio correction

Subject	Rest		3.6 minutes of exercise		6.6 minutes of exercise	
	By STD formula	By mean factor 1.007	By STD formula	By mean factor 1.01	By STD formula	By mean factor 1.01
MJB	238*	233	504	481	483	468
MM	234	232	527	513	510	508
MMH	176	171	524	513	489	486
ELM	170	168	512	509	479	482
MES	176	171	444	475	430	458
AVG	199	195	502	498	478	480

* All values for oxygen uptake are given as cc. per min.

diastolic pulmonary arterial pressures were 22, 15, and 10 mm. Hg, respectively. With exercise the same clear-cut pattern of response of the pulmonary arterial mean pressure was noted in every case (Figure 2). Within 15 seconds there was a sharp rise, the pressure reaching a peak value within 2 minutes and then remaining constant (two instances) or dropping slightly to a level which was higher than the resting control value (three instances). Following the abrupt cessation of exercise, there was a prompt fall in mean pressure to a level which was less than the resting control value. The systolic and diastolic pressures followed the same pattern, with only several minor exceptions.

Oxygen uptake

The oxygen uptake at rest varied from 108 to 128 cc. per min. per sq. m. of body surface area with a mean value of 120. Assuming a respiratory exchange ratio of 0.85, the basal metabolic rates were within normal limits in three subjects, and slightly low in two. The average basal metabolic rate was within normal limits. During exercise the oxygen uptake increased from 2 to 3 times. In each instance the oxygen uptake at 6.6 minutes of exercise was significantly less than that at 3.6 minutes.

Table III shows a comparison of oxygen uptake values obtained by substituting analytical results

TABLE IV

Some physiologic effects of exercise in normal human subjects

Subject	Arterio-venous oxygen difference (*cc./100 cc.*)			Cardiac output and cardiac index (*L./min./m² BSA*)			Pulse rate (*beats/min.*)			Stroke output and index (*cc./beat* and *cc./beat/m² BSA*)		
	Rest	3.6 *min.*	6.6 *min.*	Rest	3.6 *min.*	6.6 *min.*	Rest	3.6 *min.*	6.6 *min.*	Rest	3.6 *min.*	6.6 *min.*
MJB	4.20	6.75	6.60	5.73	7.47	7.32	95	108	110	60	69	66
				3.00	3.91	3.83				31	36	35
MM	4.32	6.70	6.35	5.45	7.86	8.04	71	98	94	77	80	85
				2.98	4.30	4.39				42	44	46
MMH	3.82	6.80	6.35	4.60	7.71	7.70	95	125	126	48	62	61
				3.22	5.39	5.38				34	43	43
ELM	4.01	7.23	7.36	4.22	7.08	6.51	100	115	109	42	62	60
				2.68	4.48	4.12				27	39	38
MES	3.88	7.85	7.65	4.54	5.66	5.62	85	117	117	53	48	48
				2.99	3.72	3.70				35	32	32
AVG	4.05	7.07	6.86	4.91	7.16	7.04	89	113	110	56	64	64
				2.97	4.36	4.28				34	39	39

Subject	Minute volume of breathing (*liters*)			Oxygen uptake (*cc./min./m² BSA*)			Ventilation equivalent		
	Rest	3.6 *min.*	6.6 *min.*	Rest	3.6 *min.*	6.6 *min.*	Rest	3.6 *min.*	6.6 *min.*
MJB	5.24	7.92	8.40	125	264	253	2.20	1.57	1.74
MM	5.02	8.50	9.44	128	288	279	2.14	1.61	1.85
MMH	3.26	8.58	8.70	123	366	342	1.85	1.64	1.78
ELM	3.44	8.75	8.51	108	324	303	2.02	1.71	1.78
MES	3.22	8.32	6.88	116	292	283	1.83	1.87	1.60
AVG	4.04	8.41	8.39	120	307	292	2.01	1.68	1.75

for carbon dioxide and oxygen concentrations in expired gas in the standard formula (9), and obtained by the use of an average respiratory exchange ratio correction (3). According to Dexter, Whittenberger, Haynes, Goodale, Gorlin, and Sawyer (3) it is not necessary to determine the carbon dioxide concentration in expired gas in order to obtain the oxygen uptake. They used a mean correction factor of 1.007 for converting expired volume to inspired volume at rest, and a factor of 1.01 for exercise. The volume of oxygen inspired was therefore calculated as percentage inspired oxygen × volume of expired gas × 1.007 or 1.01. There is a remarkable similarity of the corresponding individual values and averages for rest. For exercise, there are several discrepancies; however, the average values show excellent agreement.

Arterial blood oxygen saturation

The resting arterial oxygen saturations varied from 92 per cent to 96 per cent with a mean of 94 per cent. Samples collected at 3.6 minutes of exercise showed a slight increase in every instance. The mean arterial blood oxygen saturation at 3.6 minutes of exercise was 95 per cent.

Several factors influence the arterial oxygen saturation during exercise. Those tending to decrease it are the increased rate of pulmonary capillary flow, and the decreased venous saturation, while that tending to increase it is the increased respiratory surface area with its associated improvement of the ventilation-perfusion relationship. The latter has the effect of decreasing the slight venous admixture which is normally present. Hyperventilation, if present, would represent a second factor tending to increase arterial oxygen saturation through increased alveolar oxygen pressure. Either or both of these factors tending to increase arterial saturation could have been operative in our subjects.

Arterio-venous oxygen difference

The resting arterio-venous oxygen differences varied from 3.82 to 4.32 volumes per cent, with a mean of 4.05 (Table IV). At 3.6 minutes of exercise the arterio-venous oxygen difference had increased in every instance (mean 7.07 volumes per cent). The values for 6.6 minutes of exercise tend to be slightly less than those for 3.6 minutes.

The mean value of 4.05 volumes per cent for arterio-venous oxygen difference at rest, compares well with values previously reported (1–3, 10, 11). Our values have a very narrow range. With exercise the arterio-venous oxygen difference increased as did the cardiac output. The A-V difference exceeded 6 volumes per cent during exercise in every case.

Cardiac output

The resting cardiac output varied from 2.68 to 3.22 L. per min. per sq. m. of body surface area, with a mean of 2.97 (Table IV). At 3.6 minutes of exercise, the mean cardiac index had risen to 4.36, and at 6.6 minutes the mean value was 4.28, a difference which is not significant.

The mean value of 2.97 for resting cardiac index agrees well with published values (12, 13). This fact, together with the failure to find low values for A-V difference, would seem to indicate that anxiety did not greatly disturb the resting circulatory dynamics of our subjects (10). However, the fact that three of the five subjects had definitely elevated resting pulse rates, indicates some degree of anxiety.

Pulse rate and stroke output

The resting pulse rate varied from 71 to 100 beats per min., with a mean of 89 (Table IV). At 3.6 minutes of exercise the mean pulse rate had risen to 113, and at 6.6 minutes it was 110, a decrease which is not significant. The mean pattern of response of the pulse rate to exercise (Figure 1) exhibits a striking similarity to that of the pulmonary arterial mean pressure (Figure 2). This pattern may be described as follows: a prompt rise in pulse rate following the abrupt start of exercise, the achievement of a steady state level, and a prompt fall following the abrupt cessation of exercise to a level lower than the resting control value.

The resting stroke output varied from 27 to 42 cc. per beat per sq. m. of body surface area, with a mean of 34 (Table IV). At 3.6 minutes of exercise the mean stroke index had risen to 39 cc. per beat per sq. m., but at 6.6 minutes there had been no further appreciable change in any case, the mean value having remained 39.

In three instances the pulse rate increased relatively more than the stroke output, in one (ELM) the stroke output increased relatively more than the pulse rate, and in one (MES) the stroke output diminished slightly as the pulse rate increased. The response of ELM may be explained by the fact that this subject had the highest resting pulse rate and the lowest resting stroke output, so that during adaptation to exercise the stroke output changed relatively more. In the case of MES it would seem that in response to exercise an excessive tachycardia resulted in an actual decrease of stroke output. As will be pointed out later, the same subject (MES) responded to exercise with excessive hyperventilation.

Minute volume of breathing and ventilation equivalent

The resting minute volume of breathing varied from 3.22 to 5.24 liters (STPD), with a mean value of 4.04 (Table IV). At 3.6 minutes of exercise, the mean value had slightly more than doubled (8.41 L. per min.), and the individual values were all within 0.85 liter of each other. As exercise continued, the minute volume remained quite constant in two cases, rose slightly in two cases, and fell somewhat in one case (MES).

The resting ventilation equivalent varied from 1.83 to 2.20 with a mean value of 2.01. At 3.6 minutes of exercise, the ventilation equivalent had decreased in every instance except one (MES). All values for 6.6 minutes of exercise showed a slight increase (except MES), which in no instance reached the resting level. This increase reflects the uniform drop in oxygen uptake, and the unchanged or increased minute volume of breathing between 3.6 and 6.6 minutes of exercise. Subject MES responded to exercise with excessive hyperventilation, as shown by an initial increase of ventilation equivalent and respiratory exchange ratio. As the hyperventilation subsided from 3.6 to 6.6 minutes of exercise, the ventilation equivalent also diminished; however, the fact that the respiratory exchange ratio was still high at 6.6 minutes is evidence for persistent psychogenic hyperventilation.

The decrease in ventilation equivalent which occurs during exercise in normal subjects has been emphasized by Baldwin, Cournand, and Richards (14). Other workers (15) have noted an initial decrease, followed by a slow increase in ventilation equivalent as the steady state of exercise was approached. They also pointed out that this slow increase in ventilation equivalent was the result of diminishing oxygen uptake and the tendency of minute volume slightly to rise.

DISCUSSION

Our values for resting pulmonary arterial systolic, mean, and diastolic pressures are in close agreement with previously published values (1–3, 12, 13). In each of our five subjects the same pattern of response of the pulmonary arterial mean pressure to exercise was found: a prompt rise in pressure following the abrupt start of exercise, the achievement of a steady state level which was higher than the resting control value, and a prompt fall in pressure, following the abrupt cessation of exercise, to a level lower than the resting control value. This pattern of response to exercise has not been previously described in human subjects. On the contrary, it has been stated that the pulmonary arterial pressure does not rise appreciably with mild exercise in subjects with normal cardiorespiratory systems (1–3).

Euler and Liljestrand (16), working with anesthetized cats, noted a moderate rise of pulmonary arterial pressure during exercise in four of five experiments. The pattern of change which these workers found is identical with that which we have described in this paper.

Hickam and Cargill studied the effects of exercise on normal subjects in the supine position, and concluded that the normal pulmonary vascular bed can accommodate a large increase in the rate of blood flow with little or no increase in pulmonary arterial mean pressure (1). The work rates of their subjects were all comparable to those of ours. Their data show that in seven exercise studies on seven subjects a pressure increase was recorded in five instances (2, 5, 2, 2, and 2 mm. Hg, respectively) and a decrease in two instances (1 and 2 mm. Hg, respectively). Dexter and his coworkers, who also studied the effects of exercise on normal subjects in the supine position, concluded that only at work rates corresponding to oxygen uptakes exceeding 400 cc. per min. per sq. m. of body surface area does a rise of pulmonary arterial pressure occur (3). These authors stated that exer-

cise, producing an oxygen uptake of less than 400 cc. per min. per sq. m., was accompanied by inconstant changes of pressure in the pulmonary artery. Four of their subjects exercised at work rates comparable to those of ours, resulting in an increase from an average of 133 at rest to 288 cc. per min. per sq. m. during exercise, compared to an increase for our five subjects from an average of 120 to 307. Their data for these four subjects show that the pulmonary arterial mean pressure increased 4 mm. Hg, which is an increase of approximately one-third over their average resting control mean pressure of 13 mm. Hg. These authors (1, 3), using a cautious criterion for elevation of the pulmonary arterial pressure, hesitated justifiably to conclude that the increases which they observed were significant. Although the pressure changes which we observed are of the same order of magnitude as those referred to above, the changes reported in the present study are highly significant because of the serial nature of the observations.

It should be emphasized that our study, and those of Dexter and his associates, and Hickam and Cargill were made on subjects exercising in the supine position. Man is almost unique among mammals in his preference for the supine rather than the prone posture when recumbent. The center of mass of the left ventricle is 7 to 10 cm. higher, in relation to the center of mass of the lungs, in the supine position than when one is erect. From this consideration alone, it is obvious that the supine position is a very special posture, and that our conclusions based on studies of subjects exercising in the supine position would not necessarily apply to exercise in the erect position.

Riley, Himmelstein, Motley, Weiner, and Cournand (2) reported the effects of exercise in the sitting position on three normal subjects. In five trials on three subjects exercising on a bicycle ergometer, the pulmonary arterial mean pressure decreased four times (7, 5, 1, and 5 mm. Hg, respectively) and increased once (1 mm. Hg). These results are in contrast to those obtained during exercise in the supine position; however, it would seem important to confirm these observations through further study.

The pulmonary arterial pressure rise observed during supine exercise could be due to either or both of the following factors: increased cardiac output or increased total pulmonary resistance. Calculations from our data for 6.6 minutes of exercise indicate that the observed pulmonary arterial mean pressure rises were completely accounted for by the increases in cardiac output in every case except one (MJB), where an increase of calculated total pulmonary resistance made a slight contribution.

Although the cardiac output during the first minute of exercise is unknown, it is likely that the prompt pulmonary arterial pressure rise following the abrupt start of exercise is due to increased cardiac output. An increase of total pulmonary resistance at that time seems unlikely. The finding of increases of cardiac output which are relatively greater than the pulmonary arterial pressure increases during exercise indicates a decreased total pulmonary resistance. This was definitely the case in three of our five subjects (MMH, ELM, and MES). In these three subjects, the pulmonary arterial pressure rose during exercise to a peak value, and then descended to a steady state level. The two subjects in whom a significant decrease of total pulmonary resistance during exercise did not occur (MJB and MM), failed to show this descent from a peak value. The rate of decline of the pulmonary arterial mean pressure from its peak to a plateau may be an index of the rate at which vascular dilatation occurs, and the prompt fall following the abrupt cessation of exercise to a level lower than the resting control value suggests that the cardiac output decreases more rapidly than the pulmonary vascular bed contracts.

It has been suggested (1, 2) that this fall of total pulmonary resistance in association with increased cardiac output indicates the opening of new vascular channels or the further widening of those already open. The possibility was considered that a rise of left atrial pressure might mask a decrease in pulmonary vascular resistance. If the pulmonary "capillary" pressure accurately reflects the left atrial pressure, the data of Dexter and his associates (3) would suggest that the left atrial pressure may rise during mild, supine exercise in normal human subjects.

Comparisons have been made of the pressure-flow relationships in the post-pneumonectomy patient with those in the exercising normal subject. Our finding, that mild supine exercise results in

an increase of pulmonary arterial pressure, suggests that conditions in the resting, supine post-pneumonectomy patient are different from those in the exercising normal subject. Apparently, the acute experiment is not strictly comparable to the post-pneumonectomy situation.

With the degree of exercise employed in our study, the oxygen uptake increased from two to three times. Although the rate of oxygen uptake is an index of the work rate, several factors render this index approximate: failure to achieve the steady state, qualitative variations in protein metabolism, and the varying heat equivalent of a unit volume of oxygen depending upon the ratio of protein, fat, and carbohydrate being consumed by the body. Considering both the values for oxygen uptake during exercise and the calculated rate of performing external work, the exercise done by our subjects must be considered mild.

The use of an average respiratory exchange ratio correction factor to obtain the oxygen uptake eliminates the necessity for determination of carbon dioxide in expired gas (3). Values for oxygen uptake at rest obtained by this method were in very close agreement with those obtained by use of the conventional formula (9). Values for oxygen uptake during exercise, however, showed several discrepancies. As expected, the 3.6 minute comparisons agreed less well than those for 6.6 minutes. This is due to the fact that before the steady state is approached, there is greater variability, and therefore unpredictability, of the respiratory exchange ratio than after achievement of a semi-steady state, when the ratio is more nearly equal to the metabolic respiratory quotient. In this connection, it should be noted that Lukas and Dotter found the respiratory exchange ratio of their mitral stenosis patients to be too variable, particularly during exercise, to permit the use of mean correction factors for adjusting expired to inspired air volume (17).

The observed decrease of oxygen uptake from 3.6 to 6.6 minutes of exercise (5 per cent) reflects the gradual achievement of the steady state. Other workers (15) have also found a decrease of 5 per cent after the third minute of exercise. The difficulty of achieving a steady state during exercise, and its importance for the interpretation of data obtained during exercise, have been stressed (18). The necessity for having achieved the steady state before determining cardiac output by the Fick principle has recently been reemphasized (19). Significant changes of oxygen uptake, arterio-venous oxygen difference, and respiratory exchange ratio for expired gas between 3.6 and 6.6 minutes of exercise, indicate, as was anticipated, that our subjects had not achieved the steady state by 3.6 minutes. The length of time required to attain the steady state during exercise would be even greater for more intense work rates, or for subjects with cardiopulmonary disease.

Increased utilization of oxygen by the exercising tissues precedes the circulatory and respiratory adjustments necessary for increased oxygen supply. Bruce and his associates (15) related the peak value for oxygen uptake, which occurs by the third minute of exercise, to the increasing A-V oxygen difference, the result of diminishing venous blood oxygen content, due, in turn, to the lagging increase of cardiac output. As exercise continues and the steady state is approached, oxygen delivery to the tissues comes into equilibrium with oxygen utilization. Thus, while work rate and therefore tissue oxygen utilization remain constant, delivery and uptake of oxygen slowly decline from their peak values, A-V oxygen difference slowly decreases, and cardiac output remains relatively constant.

From the foregoing it is apparent that the Fick principle was not entirely applicable to the determination of cardiac output in our subjects at 3.6 minutes of exercise. The 3.6 minute values for cardiac output are subject to an error, which from our data is unpredictable both as to direction and magnitude. It would appear, however, from the fact that the 3.6 minute values were very nearly equal to the values at 6.6 minutes, that the 3.6 minute values as calculated were slightly in excess of the true cardiac outputs.

SUMMARY

1. Data regarding the circulatory and respiratory adaptation to exercise of five normal adults are presented and their significance is discussed.

2. The pulmonary arterial mean pressure responded in each instance with a prompt rise following the abrupt start of exercise, the achievement of a steady state level which was higher than the resting control value, and a prompt fall fol-

lowing the abrupt cessation of exercise, to a level lower than the resting control value.

3. The difficulty with which a steady state of the respiration and circulation is reached during exercise, and the necessity for having achieved the steady state prior to determination of cardiac output by the Fick principle, are emphasized.

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DISAPPEARANCE FROM SERUM OF I^{131}-LABELED L-THYROXINE AND L-TRIIODOTHYRONINE IN EUTHYROID SUBJECTS [1]

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The present report describes disappearance curves of I^{131}-labeled l-thyroxine and l-triiodothyronine. The radioactive compounds were injected intravenously in eleven euthyroid subjects, and the radioactivity of the serum was determined daily for ten to fourteen days.

MATERIALS AND METHODS

Eleven clinically euthyroid subjects were selected: two medical student volunteers, six patients with neurological disorders hospitalized for rehabilitation, one with chronic glomerulonephritis, one convalescing from a myocardial infarction, and one psychoneurotic patient without detectable organic disease. The serum butanol extractable iodines (BEI) of nine of the eleven subjects were within the normal range (1).

I^{131}-labeled l-thyroxine and l-triiodothyronine were supplied by Abbott Laboratories. Upon receipt, the triiodothyronine was purified of five per cent of contaminating thyroxine by paper chromatography with a butanol-dioxane-ammonia solvent (2) controlled by radioautography. The lots of radiothyroxine were also chromatographed but impurities were not removed.

Patients R. W., I. T., F. M., and R. K., received fifteen drops of Lugol's solution daily, while patients M. A., V. F., M. H., E. K., and O. C. and the normal volunteers were given three drops of saturated potassium iodide solution daily for two or more days prior to and throughout the course of the studies. Venous blood samples were taken ten minutes after intravenous injection of 25 to 50 μc. of the radioactive compounds (containing 1 to 6 micrograms of iodine) and then daily for ten to fourteen days. Duplicate 1 cc. aliquots of serum were dried in aluminum planchets with 0.1 cc. of one per cent detergent solution (Hemosol) to minimize cracking and flaking of the dried films.

Radioactive assay was done in a windowless flow proportional counter similar to the model designed by Robinson (3). Corrections for radioactive decay were made when necessary. Usually all samples from each patient were counted within a few hours after the end of the study.

RESULTS AND INTERPRETATIONS

The injection of radiothyroxine was followed in all instances by a relatively rapid initial fall in radioactivity of the serum (Figure 1). This was attributed to a distribution phase (or phases) and was not subjected to further analysis. After two days the serum radioactivity declined more gradually, forming a straight line when plotted semilogarithmically. This slow exponential component of the disappearance curve was interpreted as the rate of degradation of the administered radiothyroxine or replacement of tagged by untagged thyroxine, hence a measure of the turnover rate of the hormone.

From the linear component of the disappearance curve extrapolated back to zero time (Figure 1) the half-time of thyroxine turnover was obtained graphically. The turnover rate was computed from the half-time.[4] These calculations depend

[4] Calculation of Thyroxine Turnover Data (Subject M. A., Figure 1)

Half-time ($t_{1/2}$) = 5.9 days (obtained graphically from linear semilogarithmic plot. Figure 1). of thyroxine disappearance.

Turnover rate $(k \times 100) = \frac{100 \times \ln 2}{t_{1/2}} = \frac{100 \times 0.693}{5.9 \text{ days}}$

$= 11.7\%/\text{day}$

expressed as %/day. This represents the per cent of body's extrathyroidal thyroxine pool.

These relations may be derived from the equation for the exponential decay:

$$A = A_0 e^{-kt}$$

where

A = Radiothyroxine at any time
A_0 = Radiothyroxine at zero time
t = Time in days
k = Turnover rate as fraction of the body's extrathyroidal thyroxine pool turned over per day. This is conveniently expressed as per cent per day ($k \times 100$).

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upon the assumption that a steady state existed, which appears reasonable in the absence of significant alterations in the clinical condition.

The kinetic data from thyroxine and triiodothyronine disappearance curves are listed in Table I. Triiodothyronine curves had a shorter half-time and faster rate of disappearance (Figure 1) than thyroxine. Because of the small groups of patients, it is of interest to compare the successive studies with l-thyroxine and l-triiodothyronine performed seven weeks apart in subject R. K. The more rapid disappearance rate of triiodothyronine is clearly evident (Figure 2).

The "extrathyroidal thyroxine iodine pool" in micrograms was estimated from the quotient,

$$\frac{\text{total radioactivity injected}}{\text{radioactivity per microgram of BEI}};$$

the denominator was obtained from the zero time extrapolation which was taken to represent radioactivity in serum if distribution had occurred instantaneously after injection. In the two normal volunteers the pools approximated 350 micrograms of extrathyroidal thyroxine iodine (340 and 356 micrograms in W. J. and E. S., respectively). The product of these pools and the turnover rates (Table I) gave values approximating 40 micrograms of thyroxine iodine secreted and degraded daily.

The estimated pools in the nine hospitalized patients were variable; different lots of radiothyroxine gave different values, possibly owing to variable amounts of non-thyroxine radioactive organic compounds demonstrated by chromatography. Such radioactive contaminants would raise the numerator of the above quotient, but if rapidly degraded or excreted, they would not affect

TABLE I

Data from disappearance curves of I^{131}-labeled thyroxine and triiodothyronine in euthyroid subjects

Compound administered	Patient and unit No.	Age	Sex	Diagnosis	BEI micrograms % (normal 3.2–6.4)	Kinetic data: Half-time $t_{1/2}$ (*days*)	Kinetic data: Turnover rate $k \times 100$ (*%/day*)
I^{131}-tagged l-thyroxine	W. J.	24	M	Normal volunteer	4.3	6.4	10.8
	E. S.	24	M	Normal volunteer	4.1	5.6	12.4
	R. W. 39–03–33	30	M	Traumatic myelopathy Paraplegia	5.0	7.5	9.3
	I. T. 5–49–81	53	F	Hemiplegia	5.9	9.5	7.3
	M. A. 38–69–88	60	F	Hemiplegia	6.7	5.9	11.7
	V. F. B48561	35	F	Congenital myelopathy Paraplegia	6.0	5.2	13.3
	F. M. 39–00–80	63	F	Hemiplegia	3.9	6.0	11.5
	R. K. 37–77–77	46	M	Traumatic myelopathy Paraplegia	4.5	6.1	11.4
				Mean		6.5	11.0
I^{131}-tagged l-triiodothyronine	R. K. 37–77–77	46	M	Traumatic myelopathy Paraplegia	5.2	2.4	28.9
	M. H. 38–35–10	68	M	Myocardial infarction	4.4	2.2	31.5
	E. K. 39–29–24	43	F	Psychoneurosis	—*	2.5	27.7
	O. C. 39–03–90	46	M	Chronic glomerulonephritis	—*	3.2	21.6
				Mean		2.6	27.4

* Not determined because of previous intravenous pyelography.

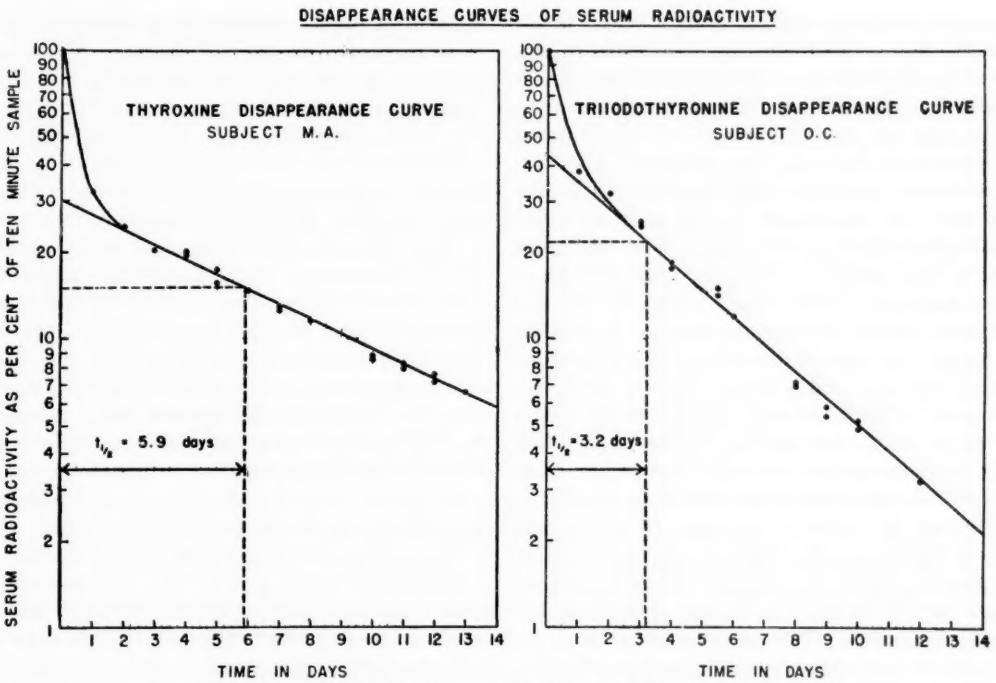

FIG. 1. PLOT OF SERUM RADIOACTIVITY (LOGARITHMIC SCALE) AGAINST TIME

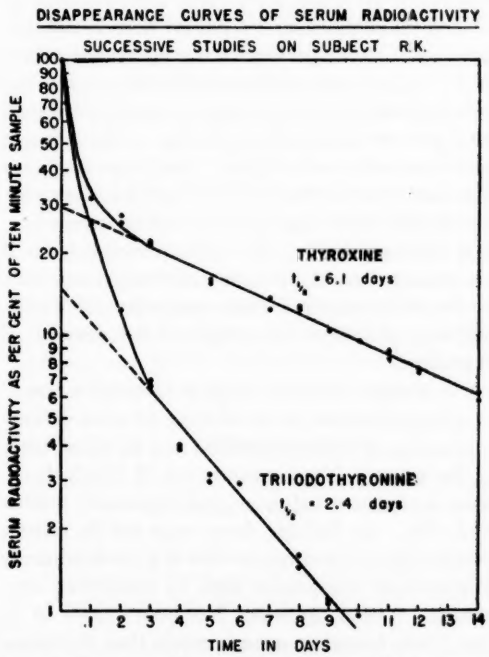
DISAPPEARANCE CURVES OF SERUM RADIOACTIVITY
SUCCESSIVE STUDIES ON SUBJECT R.K.
SERUM RADIOACTIVITY AS PER CENT OF TEN MINUTE SAMPLE
THYROXINE
$t_{1/2}$ = 6.1 days
TRIIODOTHYRONINE
$t_{1/2}$ = 2.4 days
TIME IN DAYS

FIG. 2. PLOT OF SERUM RADIOACTIVITY (LOGARITHMIC SCALE) AGAINST TIME

the denominator, thus yielding an overestimation of the pool. The slow component of disappearance should not be significantly affected. The studies with the normal volunteers were obtained with a lot of radiothyroxine purified by column chromatography (Abbott Laboratories) and checked on receipt for absence of contaminants by paper chromatography.

DISCUSSION

The "extrathyroidal thyroxine pool" is thought to represent a first approximation of the protein-bound thyroxine mainly in the extracellular fluid compartment including the plasma. It is recognized that there may be an inherent error due to inequalities of rates of thyroxine degradation in different sites. For example, the liver may remove an excessive proportion of radiothyroxine from the plasma prior to distribution in extravascular thyroxine stores, and this may depress the zero time extrapolation value. This error is probably small and may be corrected in future studies by data on excretion of the radioactive label.

The distribution of various radioactive thyroxine preparations after intravenous injection has

been the subject of previous work (4, 5). In the present report no attempt has been made to analyze the distribution of administered labeled hormone into various body compartments or sites. The data are recorded simply as disappearance curves which exhibit a slow component after distribution is complete. The semilogarithmic plot of this slow component formed a straight line throughout the study which lasted more than twice the biologic half-life. The prior administration of non-radioactive iodine was intended to minimize thyroid uptake and reutilization of radioiodine liberated on degradation of the administered labeled hormone. By simple dilution effect, the presence of larger amounts of the non-radioactive form of the element may be expected to diminish the absolute amount of radioactive iodine taken up by the euthyroid gland without necessarily influencing the output of hormone (6–8). Under these conditions, the disappearance curve should reflect peripheral degradation of labeled material and its replacement by newly synthesized unlabeled hormone. The turnover rates computed should be equal to the rates of formation and degradation, which in a steady state are identical. The injection of tracer amounts of labeled material (containing 1 to 6 micrograms of iodine) would not be expected to have an appreciable effect upon the subject's physiologic state or the amount of circulating thyroid hormone.

The validity of the turnover data depends upon the further assumption that the administered I^{131}-tagged l-thyroxine behaves *in vivo* in precisely the same fashion as the natural hormone secreted by the thyroid gland. Gordon, Gross, O'Connor, and Pitt-Rivers (9) have shown that radiothyroxine added to plasma *in vitro* is indistinguishable electrophoretically from the naturally occurring thyroxine-alpha-globulin complex. On the other hand, the data of Myant and Pochin (5) suggest possibly significant differences in the distribution phase which warrant further study. These authors suggested that the 80 microgram doses of thyroxine may have represented an excessive addition to the circulating hormone.

Biosynthesized hormone was employed in the recent paper of Hamolsky, Freedberg, Kurland, and Wolsky (10) which reported data on the disappearance of radioactivity in euthyroid, thyrotoxic and myxedematous subjects after intravenous infusion of plasma obtained from thyrotoxic patients two to six days after I^{131} therapy. The mean half-time in four euthyroid individuals was 55.7 hours (2.3 days), a much shorter half-time than the present mean of 6.7 days. Unfortunately, their observations were not extended beyond 70 hours. As they suggest, a more prolonged study might have revealed a slower component than they used in their computations. This could well account for the discrepancy. Inspection of the disappearance curves of the present report show that if no points had been obtained after the third day, it would not have been possible to plot the slow linear component which has been followed for more than two half-lives in the present data. A study in progress by Berson and Yalow using biosynthesized hormone but with observations over a two-week period (11) has thus far yielded results in general agreement with the present findings.

Since Gross and Pitt-Rivers identified triiodothyronine in human plasma (12, 13), this compound has been shown to have a potent calorigenic effect in myxedematous subjects (14). In view of the observations that it exerts its calorigenic effect more rapidly but for a briefer period than thyroxine (15–17), the more rapid disappearance of labeled triiodothyronine reported here is not unexpected. A comparison of disappearance curves of I^{131}-tagged thyroxine and triiodothyronine has been reported (15) in a single treated myxedematous patient with findings similar to the present results in euthyroid subjects. On the basis of such data and other evidence (18, 19) it has been suggested that while thyroxine bound to serum protein (alpha-globulin) (9) is the principal form of circulating thyroid hormone, triiodothyronine may be the active principle which enters the tissue cells, and may be formed by peripheral deiodination of thyroxine.

No attempt has been made to estimate a "pool" of triiodothyronine in the absence of more definite knowledge of its concentration and its significance in the serum. The concentration of triiodothyronine in plasma is unknown, but is probably minute (13, 19). Its biologic decay may not be strictly comparable to the turnover rate of a predominantly extracellular component such as circulating thyroxine. Triiodothyronine has been shown to be less firmly bound to serum protein than thyroxine (19, 20). This may account for the more rapid

disappearance from the serum. The triiodothyronine disappearance curves and the "turnover rates" derived must therefore be viewed as empirical data pending further clarification of the role of this compound in the metabolism of the thyroid hormone.

SUMMARY

1. Tracer amounts of l-thyroxine or l-triiodothyronine labeled with I^{131} were injected intravenously in euthyroid subjects.

2. The subjects received non-radioactive iodine during the studies to minimize thyroid uptake and reutilization of radioiodine liberated on degradation of the administered compounds.

3. Serum radioactivity was measured on blood samples drawn ten minutes after injection, and daily for two weeks.

4. There was a rapid initial fall, which was attributed to distribution of the compound. Within two days after injection of radiothyroxine, the serum radioactivity declined more gradually, forming a straight line when plotted semilogarithmically. This slow exponential component of the disappearance curve was interpreted as indicating thyroxine turnover, or the rates of formation and degradation, which in a steady state should be identical. The mean half-time of thyroxine turnover in six subjects was 6.7 days.

5. The disappearance curve observed after injection of labeled triiodothyronine was much more rapid, with a mean half-time of 2.7 days in four subjects. Successive studies with l-thyroxine and l-triiodothyronine in one individual clearly demonstrated the more rapid disappearance of the latter compound.

6. The interpretation of triiodothyronine disappearance curves must await further knowledge of its concentration in the serum and its precise role in the metabolism of the thyroid hormone.

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DIFFERENCES BETWEEN THE ACTIVITIES OF MATURE GRANULOCYTES IN LEUKEMIC AND NORMAL BLOOD[1]

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The abnormal appearance and number of granulocytes in the various leukemias suggest that phagocytosis by granulocytes in all stages of maturity might be so disturbed that leukemic patients might become more susceptible to bacterial infections than normal persons. Phagocytic activity among immature granulocytes is only slight but increases with maturity and becomes prominent among metamyelocytes and mature leukemic neutrophils (1–4). It has not been established, however, whether mature leukemic neutrophils are equal in phagocytic activity to normal mature neutrophils. Such a comparison has been attempted by Hirschberg (5) who concluded that the mature neutrophils in leukemic blood displayed less phagocytic activity than mature neutrophils in normal blood. Her results are open to question, however, because comparisons were made between phagocytic systems which were not uniform with respect to: 1) The number of phagocytes per unit volume; 2) quantity of opsonin; and 3) character of suspending menstruum. She also neglected to take into account the possible effect of antileukemic treatment on the phagocytic properties of leukocytes.

In the present study, the activity of mature leukemic granulocytes was measured in two ways: 1) Suspensions of leukemic and suspensions of normal leukocytes, containing approximately equal numbers of mature granulocytes, were inoculated with opsonized type II pneumococcus and the phagocytic activity of leukemic granulocytes was compared with that of normal granulocytes. 2) Heparinized leukemic blood was divided into two portions and the buffy coat removed from one to lower the count of mature neutrophils to normal levels; each portion was inoculated with pathogenic staphylococci and the number of culturable staphylococci were counted before and after six hours' incubation.

The simultaneous performance of these two tests on specimens from a given patient provided information not only on the action of single mature granulocytes in a standardized menstruum, but also on the collective action of leukemic leukocytes in their own blood. Observations were also made: 1) On the effect of antileukemic therapy on phagocytosis by neutrophils; and 2) on the incidence of bacterial infection in the patients whose leukocytes were examined.

METHODS AND MATERIALS

1. *Clinical material.* Patients were studied at the University Hospital or the Simpson Memorial Institute of the University of Michigan. The diagnosis of leukemia was established in each case by staff members of the Simpson Institute through laboratory examination of peripheral blood and bone marrow and clinical examination of the patient. The number of cases of each type of leukemia and the treatments are listed in the protocol. Roentgen irradiation was administered at the University Hospital under the supervision of Dr. Isadore Lampe.

Freshly drawn samples of venous blood obtained from these patients were divided into two portions. One portion was used to provide leukocytes for the test with opsonized pneumococci and one portion was inoculated with pathogenic staphylococci as described below.

2. *Measurement of leukocyte activity in a standard phagocytic system.* Leukocytes from patients with small buffy layers and from normal persons were separated from fresh venous blood (containing heparin 0.5 mg. per ml. as anticoagulant) by permitting the erythrocytes to sediment at 37° C. for 30 minutes in a glass tube tilted at 60 degrees; then the supernatant plasma was removed with a warm sterile pipette. Supernatant plasma obtained in this way usually contained at least as many leukocytes per cu. mm. as existed in the whole blood, but almost no erythrocytes. Sometimes it was necessary to centrifuge the supernate or the blood itself at 500 r.p.m for 5 minutes to remove the erythrocytes. The leukocytes were sedimented only slightly at this speed. At first, only siliconized glassware was used, but later it was

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TABLE I

Antibacterial action of leukemic whole blood and of suspensions of leukemic leukocytes from patients with chronic granulocytic leukemia *

CHRONIC GRANULOCYTIC LEUKEMIA

NAME AGE DURATION	DATE	WBC X1000	PERIPHERAL BLOOD DIFFERENTIAL S	B	I	O	MATURE NEUTROPHILS PER MM3	CULTURABLE STAPHYLOCOCCI (COLONIES PER ML.) BEFORE	AFTER 6 HOURS	RINGERS SUSPENSION OF LEUKOCYTES APPROXIMATELY 14,000 MATURE NEUTROPHILS (BANDS AND SEGS) PER MM3 PER CENT OF MATURE NEUTROPHILS PHAGOCYTOSING PNEUMOCOCCI S/B	PATIENT	CONTROL	AVERAGE NUMBER BACTERIA PER PHAGOCYTIC CELL PATIENT	CONTROL	TREATMENT
R.F. 57 8 mo.	3/24/53	476.	13	9	73	5	105,000	16,800	106	90/10	11	100	7.0	10.5	X-Ray to spleen begun this date
	3/27/53	364.	30	14	54	2	160,000	18,000	3,600	55/45	25	100	3.2	8.1	3rd day of X-Ray to spleen
	4/2/53	148.	52	33	8	2	125,000	18,600	848	48/52	78	100	2.8	14.5	1 week after X-Ray
M.W. 39 10 mo.	3/19/53	8.	50	0	0	50	4,000	22,000	4,400	100/0	100	100	16.0	16.0	Sustained response to P32 given July 1952 when WBC was 99,000
M.R. 21 4 mo.	4/24/53	410.	18	39	39	4	240,000	4,800	1,560	60/40	48	100	8.9	3.8	None
	4/29/53	370.	21	14	55	10	130,000	20,000	694	80/20	4	90	10.0	9.0	Urethane started 4/28/53
	5/5/53	379.	35	29	33	3	255,000	18,000	648	55/45	18	46	4.5	4.7	1 week of urethane
	5/11/53	338.	45	23	30	2	230,000	9,600	32	20/80	11	100	0.7	9.5	Urethane stopped preceding day
E.J. 6 mo.	12/15/52	430.5	29	33	26	12	275,000	21,000	1,018		38	100	3.3	9.0	
	12/23/52	222.	28	33	31	8	140,000				74	100	13.5	17.7	P32 on 12/17/52
T.B. 26 6 mo.	1/12/53	85.	51	6	33	10	49,000			67/33	86	87	9.9	23.3	P32 July, August, October, and December 1952
	2/9/53	143.	48	14	28	10	92,000	18,000	688	84/16	26	100	9.6	10.5	P32 this date
	4/10/53	62.	54	26	10	10	50,000	18,800	1,640	88/12	60	90	5.0	10.7	Total body irradiation 3/30/53 to 4/10/53
A.E. 3 yr.	2/20/53	19.	76	0	10	14	15,000	20,600	12,000	100/0	21	86	1.3	20.1	No recent treatment
	3/6/53	11.	72	2	0	26	9,000	22,000	6,200	95/5	95	95	16.2	16.6	X-Ray to spleen 2/20/53 to 3/6/53 (8 wk.)
M.P. 55 9 mo.	1/12/53	730.	16	26	55	1	300,000	13,680	276	64/36	15	100	19.5	25.0	None
	1/14/53	800.	18	34	65	3	400,000	19,600	588		0	90	0.0	15.0	
M.P.	3/31/53	37.	9	13	42	38	8,100	18,000	6,000	60/40	86	94	16.5	13.0	Total body X-Ray started 3/26/53 and completed 4/8/53
M.S. 51 3 yr.	4/29/53	340.	28	8	71	5	115,000	18,800	580	40/60	15	89	3.0	9.0	Received 10 mg. TEM on day before tests
C.S. 46 3 yr.	3/20/53	82.	45	18	23	14	51,000	20,160	940	90/10	50	100	5.0	15.0	P32 3/19/53
E.S. 56 2 yr.	8/12/52	23.	58	17	15	20	14,900				80	83	6.6	10.3	P32 1/14/52
F.O. 45 2 yr.	7/7/52	39.7	38	52	1	9				75/25	69	96	11.1	12.4	No recent therapy
	7/11/52	25.1	35	50	4	11				65/35	55	85	5.5	8.0	Total body X-Ray started 7/9/52
	9/16/52	140.	5	0	95	0	7,000	18,360	162						Receiving total body X-Ray 9/10/52 to 9/29/52
	9/25/52	151.5	6	3	91	0	13,600	28,080	3,840						Receiving total body X-Ray
	10/3/52	187.	1	3	96	0	7,500	28,000	5,960						
H.W. 51 8 mo.	7/28/52	100.	40	16	39	5				70/30	5	44	14.5	7.3	TEM 5 mg. daily July 24, 25, 26, 1952
	10/16/52	72.5	48	16	32	6	45,000	25,800	7,600						
M.L. 53 2 yr.	12/15/52	174.5	36	31	37	6	110,000	21,600	8,400	50/50	39	100	9.0	14.0	Receiving total body X-Ray started on 12/10/52
	1/30/53	174.	29	28	35	8	102,000	22,000	9,000	40/60	52	82	4.0	7.0	No treatment - last X-Ray completed 8 weeks before
	2/4/53	209.	57	23	20	10	120,000	18,000	6,600						
	3/17/53	288.5	31	28	40	1	182,000	18,240	970	40/60	40	100	4.0	25.0	X-Ray therapy to Rt. humerus 2/11/53 to 2/17/53
	3/19/53	220.	22	22	56	0	97,000	17,800	2,600	50/50	15	82	3.0	4.2	X-Ray to spleen started previous day
	3/31/53	18.5	45	32	16	7	12,700	19,800	8,400	80/10	87	94	7.0	13.0	X-Ray to spleen completed 1 week previously
J.D. 62 26 yr.	10/13/52	197.	34	31	30	5	130,000	10,800	80						P32 9/5/52
	10/15/52	183.	32	16	52	0	81,000	18,000	1,080						TEM 3 mg. this date, also 10/16/52 and 10/17/52
	10/21/52	83.	47	26	24	3	65,000	24,600	600						
G.G. 3 mo.	12/4/52	45.5	50	10	23	17	27,000	28,000	13,200						P32 12/3/52
L.G. 42 4 mo.	11/24/52	114.	24	18	38	20	46,000	24,240	6,600						None
	12/2/52	113.5	40	5	53	2	50,000	1,000	12						Receiving urethane 3 gm. daily
	2/4/53	129.	21	18	37	24	52,000	18,000	1,568						Receiving urethane
W.H. 33 1 mo.	9/20/52	282.	31	17	42	10	140,000	18,480	846						None
	10/27/52	24.8	40	22	14	24	16,000	13,200	4,080						P32 on 9/21/52
N.D. 59 3 yr.	3/11/53	80.	40	3	18	39	33,000	19,200	5,000						X-Ray to spleen 2/27/53 to 3/8/53
E.H. 48 2 yr.	5/29/52	83.4	7	51	42	0	48,000	18,308	18,758						X-Ray to spleen 4/10/52 to 4/18/52

* Whole bloods were inoculated with pathogenic staphylococci, and leukocyte suspensions with opsonized type 2 pneumococci (S = segmented neutrophils, B = band neutrophils, I = immature neutrophils, O = leukocytes other than neutrophils; S/B = ratio of segmented to band forms.)

found that comparable results were obtained with clean glassware which had not been treated with silicone. Leukocytes from patients with high counts were easily separated by pipetting the thick buffy coat from heparinized venous blood. The plasma suspensions of leukocytes were centrifuged at 1,000 r.p.m. for 5 minutes. The supernatant plasma was then removed and replaced by Ringer's solution containing 0.5 mg. heparin per ml. The Ringer-heparin solution was added in amounts necessary to give a final count of approximately 8,000 mature neutrophils per cu. mm. No attempt was made to remove the immature neutrophils also present in the suspension. Mature neutrophils were defined as band forms and segmented forms. Immature neutrophils were defined as metamyelocytes, myelocytes, progranulocytes, and myeloblasts. All cells were identified according to the criteria reported by the committee for clarification of the nomenclature of cells (6).

A type II pneumococcus, highly virulent for mice and containing a large capsule rendering it resistant to phagocytosis in the absence of specific opsonin *in vitro*, was subcultured on rabbit blood-agar plates. At the time of each test, freshly grown cultures of the pneumococcus were removed from the surface of the plate with a platinum wire and the bacteria suspended in Ringer's solution to give a density equal to that of the No. 1 tube of the MacFarland nephelometer. The pneumococcal suspension was distributed in 0.1 ml. quantities among six glass test tubes measuring 15 × 100. Rabbit sera containing high titers of specific type II pneumococcal antibody were used to opsonize the pneumococcus by adding 0.02 ml. of antiserum to each of two tubes containing pneumococcal suspension. Pneumococci in two other tubes were similarly treated with antiserum diluted in Ringer's solution; those in the other two tubes with normal non-immune rabbit serum. The dilute antiserum was used to cover the possibility that diminished phagocytic activity of leukemic cells might not be apparent in the presence of excess antibody. The mixtures of serum and pneumococci were incubated in a water bath at 37° C. for 30 minutes. Then 0.1 ml. of leukemic cells suspended in Ringer's was added to one of each of the three pairs of

TABLE II

Antibacterial action of leukemic whole blood and suspensions of leukemic leukocytes from patients with acute and subacute granulocytic leukemia *

ACUTE AND SUBACUTE GRANULOCYTIC LEUKEMIAS

NAME AGE DURATION	DATE	PERIPHERAL BLOOD								RINGERS SUSPENSION OF LEUKOCYTES: APPROXIMATELY 8,000 MATURE NEUTROPHILS (BANDS AND SEGS) PER MM³				TYPE OF GRANULOCYTIC LEUKEMIA	TREATMENT
		WBC X1000	Differential				MATURE NEUTROPHILS PER MM³	CULTURABLE STAPHYLOCOCCI (COLONIES PER ML.)		PER CENT OF MATURE NEUTROPHILS PHAGOCYTOSING PNEUMOCOCCI		AVERAGE NUMBER BACTERIA PER PHAGOCYTIC CELL			
			S	B	I	O		BEFORE	AFTER 6 HOURS	PATIENT	CONTROL	PATIENT	CONTROL		
D.W. 22 5 mo.	7/5/52	6.1	11	0	65	24				75	82	6.7	11.1	SA;SL	None
E.L. 73 3 wk.	3/6/53	15.6	39	1	1	59	6,000	22,200	4,800	92	95	11.5	16.6	SA;SL	None
E.T.	3/5/53	3.96	59	9	11	30	2,400	20,000	150	51	60	3.8	3.0	SA;SL	?
I.B. 22 4 mo.	11/15/52	5.6	16	3	81	0				77	82	4.5	7.0	SA;SL	None
G.S. 69 5 mo.	3/24/53	227.	20	2	62	16				68	100	4.1	10.5	Acute	None
R.V. 55 9 mo.	3/19/53	32.5	22	6	70	2				98	82	9.9	4.2	Acute	Cortisone
F.S. 17 2 wk.	3/31/53	4.5	18	3	38	41	900	20,400	13,200	100	100	40.0	10.0	Acute	None
G.M. 25 4 mo.	8/15/52	11.	15	4	74	7				58	44	5.3	6.0	Acute	ACTH 25 mg. for 2 days
	10/13/52	49.75	11	6	73	10	8,500	18,000	4,800						None
	10/15/52	127.	6	2	90	2	10,000	29,000	6,000						None
	10/21/52	60.5	8	4	88	2	6,000	24,880	14,400						None
F.H. 76 6 mo.	10/23/52	17.	28	36	19	17	11,500	24,000	2,400					SA;SL	
P.C. 58 7 mo.		26.5	16	14	4	66	7,900	28,000	17,600	97	88	15.0	8.0	SA	
F.C. 59 1 mo.	1/26/53	145.	2	1	91	6	4,600	24,000	17,600						Cortisone 75 mg. - 6 H.

* (S.A. = Subacute, SL = Subleukemic; abbreviations of cells same as Table I; L = Lymphocytes).

TABLE III

Antibacterial action of leukemic whole blood and suspensions of leukocytes from patients with chronic lymphocytic leukemia *

CHRONIC LYMPHOCYTIC LEUKEMIA

NAME AGE DURATION	DATE	PERIPHERAL BLOOD								RINGERS SUSPENSION OF LEUKOCYTES: APPROXIMATELY 8,000 MATURE NEUTROPHILS (BANDS AND SEGS) PER MM³				TREATMENT
		WBC X1000	S	B	I	L	MATURE NEUTROPHILS PER MM³	CULTURABLE STAPHYLOCOCCI (COLONIES PER ML)		PER CENT OF MATURE NEUTROPHILS PHAGOCYTOSING PNEUMOCOCCI		AVERAGE NUMBER BACTERIA PER PHAGOCYTIC CELL		
								BEFORE	AFTER 6 HOURS	PATIENT	CONTROL	PATIENT	CONTROL	
W.Y. 48 1 mo.	11/21/52	128.	1	0	0	99	1,280	26,400	13,200	85	46	5.1	12.2	None
J.P.	4/24/53	352.5	<1	0	0	99+	<3,500	23,400	16,800	100	100	20	12	X-Ray to spleen 4/23/53 to 5/5/53
H.C. 74 7 yr.	12/2/52	24.	23	0	1	76				100	100	10.9	7.2	Receiving X-Ray to nodes 11/3/52 to 12/13/52
J.H. 67 2 yr.	3/5/53	400.	1	0	0	99	4,000	23,400	14,400					X-Ray to regional node 2/23/53 to 3/5/53
	4/17/53	280.	3	0	0	97	8,400	18,600	6,600	76	92	9.5	8.6	6 weeks after completion of X-Ray
A.V.	3/31/53	51.	9	0	0	91	4,590	17,000	9,600	88	94	17.6	13	
J.M. 61 2 mo.	3/3/53	186.	8	0	0	92	15,000	19,600	8,160	82	100	15	15	X-Ray to nodes 2/25/53 to 3/9/53
A.R. 63 24 yr.	12/1/52	8.7	41	0	0	59	3,600	25,600	2,640	100	100	>40	15	None within 6 months
J.O. 84 2 yr.	4/21/53	15.3	24	0	2	74	3,500	18,144	14,994					Total body X-Ray 4/17/52 to 4/25/52

* Abbreviations same as Tables I and II.

TABLE IV

Antibacterial action of leukemic whole blood and suspensions of leukocytes from patients with acute and subacute lymphocytic leukemia *

ACUTE AND SUBACUTE LYMPHOCYTIC AND MONOCYTIC LEUKEMIA

SB: Mature neutrophils
M: Monocytes
PM: Promonocytes
B: Blasts
L: Lymphocytes
I: Innumerable

NAME AGE DURATION	DATE	PERIPHERAL BLOOD									RINGERS SUSPENSION OF LEUKOCYTES: APPROXIMATELY 8,000 MATURE NEUTROPHILS (BANDS AND SEGS) PER MM³				TYPE OF LEUKEMIA	TREATMENT
		WBC X1000	SB	M	PM	B	L	MATURE NEUTROPHILS PER MM³	CULTURABLE STAPHYLOCOCCI (COLONIES PER ML.)		PER CENT OF MATURE NEUTROPHILS PHAGOCYTOSING PNEUMOCOCCI		AVERAGE NUMBER BACTERIA PER PHAGOCYTIC CELL			
									BEFORE	AFTER 6 HOURS	PATIENT	CONTROL	PATIENT	CONTROL		
H.T. 34 2 mo.	4/15/53	120.	0	0	50	43	7	0	18,000	21,600	0 Promon. active	100	I	15	Acute Monocytic	None
	4/17/53	51.	0	0	25	66	9	0	12,000	9,600	0 Promon. active	100	I	12.5		
	4/29/53	12.	0	0	30	64	6				0-Promon. active	89	I	9.		
C.K.	5/5/53	1.2	2	0	0	30	69	24	368	2,400					Subacute Subleukemic Monocytic	None
W.S. 58 2 wk.	9/26/53	5.35	17	13	10	38	22	920 +650 mono-cytes	738	348					Acute Subleukemic Monocytic	None
A.Gh	4/10/53	19.	3	0	0	0	97	570	824	11,600	90	90	33	10.7	Subacute Subleukemic Lymphocytic	None
A.G. 80	12/23/52	2.1	8	0	0	0	92	168	1,368	3,600					Subacute Subleukemic Lymphocytic	None

tubes and 0.1 ml. of normal cell suspension to each of the corresponding three tubes. All tubes were sealed with paraffin and the mixtures of leukocytes and opsonized pneumococci were rotated in a machine at 6 r.p.m. for 15 minutes. The tubes were opened and a drop of the contents spread on a glass coverslip. The film was dried, stained with Wright's Stain, and mounted on a glass slide. Phagocytic activity was determined as follows:

Approximately 100 mature neutrophils were counted; the number which had ingested pneumococci were re-

corded and subsequently referred to as "Per cent phagocytosis." The total number of intracellular pneumococci were also counted and the average number of pneumococci ingested were recorded for those cells which contained intracellular organisms. Results in Tables I, II, III, and IV are given for undiluted opsonin because the results with diluted opsonin were confirmatory.

3. *Measurements of the antibacterial action of whole leukemic blood.* The purpose of this experiment was to determine the capacity of whole leukemic blood inoculated with pathogenic staphylococci to lower the bacterial count after 6 hours incubation at 37° C. Pathogenic staphylococci were selected as the test organism not only because they produced most bacterial infections in leukemic patients, but also because they multiplied rapidly in fresh plasma or in leukocyte-free whole blood incubated at 37° C. Although this test has been regarded as a measure of bactericidal activity, it has been demonstrated that the decrease in number of bacterial colonies results from phagocytosis and intracellular clumping, rather than from killing of staphylococci (7). When leukemic or normal phagocytes are disrupted with distilled water or by raising the pH above 10, the dispersed cocci grow separately instead of in clumps and the number of colonies increases greatly in the agar plates. The alkaline pH also breaks up the extracellular clumps of coagulase positive staphylococci which are present in plasma.

The strain of staphylococcus (B) used in all experiments had been isolated from human empyema fluid. It clotted human plasma, clumped instantly in fibrinogen solution, and on blood agar produced hemolysis and a golden yellow pigment. It was maintained in a smooth phase by frequent subculture on blood agar. For routine tests, the staphylococci were grown for 18 hours in tryptose phosphate broth and then resuspended in saline to give a density equal to that of the No. 1 tube of the MacFarland nephelometer. The suspension of staphylococci was then diluted in saline either to 10^{-4} or 10^{-5} depending on whether heavy or light inoculums were desired. Because of the large errors involved in reaching a given dilution by pipetting, numbers of bacteria were selected for inoculation that could be counted in agar plates without serial dilution.

Leukemic whole blood used in these experiments was part of that drawn to provide leukocytes for the standardized phagocytic system in Section 1 and permitted simultaneous performance of both procedures on aliquots from the same sample. The portion used for inoculation with staphylococci was divided into equal aliquots; the leukocyte count was lowered considerably in one of the aliquots by pipetting off the buffy coat. Two and one half ml. of blood with buffy coat intact and 2.5 ml. of blood with buffy coat removed were each inoculated with 0.5 ml. of staphylococcal suspension and incubated for 6 hours at 37° C. in tubes measuring 38 × 180 mm. The tubes were not agitated because it was found that agitation for a

TABLE V

Antibacterial action of leukemic whole blood measured by its ability to reduce the number of culturable staphylococci (coagulase positive) during 6 hours incubation: Effect of lowering to approximately normal the numbers of mature granulocytes by removing the leukemic buffy coat (Compare with Table VA which gives results for normal blood)

Patient	Number mature granulocytes (segs. and bands) per mm³	Buffy coat removed	Staphylococci cultured before and after 6 hours incubation in leukemic whole blood (colonies per ml.)			
			Large inoculum		Small inoculum	
			Before	After 6 hours	Before	After 6 hours
R. F. 3/24/53	160,000	no	18,000	3,600	790	8
	7,000	yes	22,000	26,000	712	810
	0	Plasma			840	9,600
4/2/53	125,000	no	18,600	828	1,170	10
	9,000	yes	16,400	16,800	546	650
		Plasma	20,000	Innumerable		
E. J. 12/15/52	275,000	no	21,000	10,000	516	10
	6,500	yes	30,000	32,400	1,058	1,640
		Plasma	unable	to obtain	cell-free	plasma
L. G. 11/24/52	46,000	no	24,240	6,600	1,376	112
	10,500	yes	24,480	20,800	1,440	634
		Plasma			1,200	18,000
M. L. 3/17/53	162,000	no	18,240	970	620	8
	4,200	yes	16,800	21,000	658	1,336
		Plasma			1,024	16,400
3/31/53	12,700	no	19,800	8,400	920	288
	4,000	yes	18,000	18,000	1,058	1,158
		Plasma			1,018	2,400
A. A. E. 2/20/53	15,000	no	20,600	12,000	1,008	330
	5,700	yes	20,800	19,700	1,188	4,000
		Plasma			1,018	20,000
M. P. 1/12/53	300,000	no	13,680	476	652	28
	26,000	yes	29,400	20,400		
		Plasma	unable	to obtain	cell-free	plasma

TABLE VA

ANTIBACTERIAL ACTION OF NORMAL BLOOD MEASURED BY ITS ABILITY TO REDUCE THE NUMBER OF CULTURABLE STAPHYLOCOCCI COAGULASE POSITIVE DURING 6 HOUR INCUBATION

		NEUTROPHILS PER MM3	STAPHYLOCOCCI CULTURED BEFORE AND AFTER 6 HOURS INCUBATION IN NORMAL WHOLE BLOOD OR PLASMA (COLONIES PER ML.)			
			LARGE INOCULUM		SMALL INOCULUM	
			BEFORE	AFTER 6 HOURS	BEFORE	AFTER 6 HOURS
1.	Blood	5,500	24,000	3,880	840	52
	Plasma	0			768	6,960
2.	Blood	5,000	20,400	6,600	1,096	130
	Plasma	0				
3.	Blood	4,500	18,000	6,240	912	224
	Plasma	0			1,152	22,000
4.	Blood	6,500	17,600	3,400	260	24
	Plasma	0	10,000	40,000	364	9,840
5.	Blood	4,500	21,000	10,200	456	120
	Plasma	0			232	9,000
6.	Blood	5,500	24,400	10,320	1,440	24,000
	Plasma	0				
7.	Blood	5,000	20,400	3,600	558	56
	Plasma	0	3,840	9,000	686	2,400
8.	Blood	3,000	22,800	7,200	1,080	368
	Plasma	0			692	14,400
9.	Blood	6,300	23,000	1,200	860	16
	Plasma	0	20,400	49,000	428	6,480
10.	Blood	3,500	20,400	4,200	496	58
	Plasma	0	20,000	39,600	500	3,100
11.	Blood	5,300	22,000	2,400	672	42
	Plasma	0			424	5,200
12.	Blood	3,600	12,000	3,600	440	272
	Plasma	0	9,600	40,000	364	7,840

period as long as 6 hours was very destructive to the leukocytes. Before incubation, and immediately after removal from the incubator, the tubes were shaken to disperse the leukocytes and bacteria, and 0.5 ml. of the blood was removed each time and incorporated into agar pour plates and incubated at 37° C. for 24 hours. The bacterial colonies were then counted. Normal whole blood was inoculated with staphylococci and treated in the same way. Results were not recorded if heavy growth failed to develop in cell-free plasma controls.

4. *Phagocytic activity of normal leukocytes in leukemic plasma.* The phagocytic system described in Section 2 was useful for demonstrating an antileukocyte factor in leukemic plasma. The procedure was identical except that normal leukocytes were resuspended in leukemic plasma instead of Ringer's solution. The leukemic and normal control plasmas had been collected on the dates indicated in Table VI and stored at 4° C. until 5/19/53 and 5/21/53 when they were removed for the experiments. In this way all plasmas were tested simultaneously against normal leukocytes obtained from a given sample of blood.

RESULTS

Chronic granulocytic leukemia

Pneumococci were phagocytosed by fewer mature leukemic neutrophils than by normal neutrophils in most experiments. Phagocytosis equal to that of the normal control was observed among leukocytes from only 2 of 14 leukemic patients (A. E. 3/6/53 and M. W.) at a time when both had experienced marked clinical and hematologic remission from successful therapy. In two other cases (T. B. 1/12/53 and E. S. 8/12/53) the per cent phagocytosis by mature neutrophils approached normal, but the average number of intracellular pneumococci per phagocytic cell was markedly lower than normal. Phagocytes from 7 of the 14 patients contained at one or more times an average number of intracellular pneumococci equal to or greater than the average observed in

normal phagocytes serving as controls. Two of these patients were those who had undergone remission after treatment and whose leukocytes were all as active as normal. Only a fraction of the leukocytes from the other five patients were phagocytic, but the phagocytic cells were, on the average, as active as normal or more so. In addition to the two whose leukocyte function was normal during remission, three patients (M. L. 3/31/53, E. J. 12/23/53, R. F. 4/2/53) and perhaps a fourth (T. B. 4/10/53) manifested improvement of leukocyte function after treatment with x-ray or P_{32}. A sharp fall in phagocytic activity was observed in leukocytes from patient (M. R.) after the start of treatment with urethane.

Both band and segmented cells were regarded as mature neutrophils. Numerous reports have disclosed that there is no significant difference between the phagocytic activity of band forms and segmented forms from normal persons (2–4, 8). Phagocytosis by metamyelocytes was occasionally observed but rarely if ever occurred among myelocytes or younger cells in our preparations. Distinction between band and segmented forms was sometimes difficult when the cell contained numerous cocci. For this reason no attempt was made to compare quantitatively the relative phagocytic power of band cells and segmented cells. Table I discloses that in chronic granulocytic leukemia the per cent phagocytosis was usually much less than the per cent of segmented neutrophils in a given suspension. It is clear, therefore, that phagocytosis by segmented neutrophils was impaired in chronic granulocytic leukemia.

Samples of blood from each of the 12 patients whose mature neutrophils showed impaired phagocytosis of pneumococci reduced the number of culturable staphylococci in 6 hours as readily as normal blood. Bloods from a total of 19 of 20 patients lowered the total staphylococcal count as effectively, or more so, than normal blood. The one exception (E. H.) was a patient whose blood contained mostly band neutrophils. Although his blood did not lower the bacterial count, it prevented growth.

The observed impairment of phagocytosis by individual neutrophils in chronic granulocytic leukemia, as measured by the phagocytosis of pneumococci, was also demonstrated when the total neutrophil count of whole leukemic blood was reduced to normal. Thus, in bloods from seven patients, removal of the buffy coat not only reduced the neutrophil count to levels approaching normal, but also rendered the blood incapable of lowering significantly the count of staphylococci (Table V).

Acute and subacute granulocytic leukemia

The per cent phagocytosis by mature neutrophils was at least normal or nearly normal, in eight of nine patients (Table II). In five of these eight leukemic patients, the average number of pneumococci per phagocytic cell was normal or greater. Only in the suspension made from the cells of one patient (G. S.) were both the per cent phagocytosis and average number of intracellular bacteria reduced when compared to the normal control. He was the only patient of the nine whose total leukocyte count in the peripheral blood was greatly elevated when the test was performed. In three patients (P. C., F. S., and R. V.) there was a great increase in phagocytic activity over normal as judged both by per cent phagocytosis and number of bacteria per cell. Neutrophils from patient F. S. were so heavily packed with pneumococci that the bacteria could not be counted. All bloods from patients with acute or subacute granulocytic leukemia lowered the count of inoculated staphylococci as well as normal blood. The blood from two patients reduced the number of culturable staphylococci despite the presence of only 2,400 (patient E. T.) and 900 (patient F. S.) mature neutrophils per cu. mm.

Chronic lymphocytic leukemia (Table III)

The per cent phagocytosis by mature neutrophils from five patients was essentially normal. The per cent phagocytosis by leukocytes from two patients (J. H. and J. M.) was reduced, but not by much. Phagocytes from five of the seven patients contained more intracellular pneumococci than normal, from one patient (J. M.) the same as normal, and only those from patient W. Y., less than normal. Two of the patients (J. P. and H. C.) whose leukocytes were more active than normal were receiving x-ray therapy during the periods when the tests were made. Patient J. M., whose leukocyte function was slightly reduced, was also receiving x-ray therapy.

Although the count of mature neutrophils was 4,000 per cu. mm. or less in the blood of five of seven patients, normal or nearly normal reduction in numbers of inoculated staphylococci was observed in each case. The blood of patient A. R., containing only 3,600 mature neutrophils per cu. mm., reduced the number of culturable staphylococci by almost 90 per cent or more than that produced by normal blood. His leukocytes were also exceedingly active in the standardized phagocytic system against pneumococci.

Acute and subacute monocytic and lymphocytic leukemias (Table IV)

It was possible to test the neutrophils of only one patient (A. G.) for phagocytosis of pneumococci. These were more active than normal. Some of the promonocytes (per cent not determined) of patient H. T. showed tremendous phagocytic activity and engulfed so many pneumococci that the bacteria could not be counted in each cell. These phagocytic promonocytes were apparently so active that they restrained the growth of inoculated staphylococci and even lowered the count on one occasion (4/17/53). Similar activity of monocytes or promonocytes may be evident in the case of W. S. whose blood reduced a small inoculum of staphylococci by nearly 50 per cent. The number of phagocytes in the bloods of the other three patients in this group were too low to restrain staphylococcal multiplication.

Phagocytic activity of normal leukocytes in leukemic plasmas (Table VI)

The plasmas obtained from patient M. L. (chronic granulocytic) and from patient F. S. (acute granulocytic) abolished the phagocytic activity of normal leukocytes obtained from two donors. These plasmas also damaged the neutrophils, producing severe lysis (plasma M. L.) or pyknosis (plasma F. S.). The plasmas of three other patients (A. G., subacute subleukemic

TABLE VI

PHAGOCYTIC ACTIVITY OF NORMAL NEUTROPHILES SUSPENDED IN LEUKEMIC PLASMAS

All plasmas were stored at 4° C. and later tested simultaneously with each sample of normal cells.

SOURCE OF LEUKEMIC PLASMA			PER CENT OF NEUTROPHILES SUSPENDED IN LEUKEMIC PLASMA WHICH PHAGOCYTOSED OPSONIZED TYPE 2 PNEUMOCOCCI		APPEARANCE OF CELLS
CASE	DATE	TYPE OF LEUKEMIA	NORMAL CELLS J.F.	NORMAL CELLS A.B.	
A.G.	4/10/53	Subacute subleukemic lymphocytic	72	50	Lysis (of some cells)
M.L.	3/31/53	Chronic granulocytic	0	0	Lysis of all cells
F.S.	3/31/53	Acute granulocytic	2	0	Pyknosis of all cells
H.T.	4/29/53	Acute monocytic	64	62	Normal
A.E.	3/6/53	Chronic granulocytic	50	65	Normal
E.L.	3/6/53	Subacute subleukemic granulocytic	68	86	Normal
J.P.	4/24/53	Chronic lymphocytic	64	88	Normal
U.Y.	2/24/53	Chronic lymphocytic	65	87	Normal
M.W.	3/19/53	Chronic granulocytic	75	70	Normal
M.R.	5/5/53	Chronic granulocytic		90	Normal
M.S.	4/29/53	Chronic granulocytic		90	Normal
Normal Control. A				90	
Normal Control. B				96	
Normal Control. C			100		

TABLE VII

INCIDENCE OF BACTERIAL INFECTIONS IN LEUKEMIC PATIENTS

Infections were all staphylococcal and consisted of mild furuncles, pharyngitis, and a generalized pustular dermatitis.

TYPE OF LEUKEMIA	TOTAL NUMBER OF CASES	TOTAL NUMBER WITH PROVEN BACTERIAL INFECTION
Chronic Granulocytic	24	2
Acute Granulocytic	8	3
Chronic Lymphocytic	10	0
Acute Lymphocytic	2	0
Acute Monocytic	3	0
TOTAL	47	5

lymphocytic; H. T., acute monocytic; A. E., chronic granulocytic) also seemed to reduce phagocytosis of normal leukocytes, but not by much. The plasma of A. G. produced slight cytolysis.

DISCUSSION

There are at least two possible explanations for the decreased phagocytic activity of mature neutrophils in chronic granulocytic leukemia, observed in this study. One possible explanation is that there is an inherent defect in the leukemic cell. The other is that leukemic plasma contains a factor which is injurious to the neutrophil. Whatever the cause, the decreased phagocytic activity could not have been anticipated by morphologic study because the nuclear configuration and cytoplasmic structure of most of the mature leukemic neutrophils were normal. The existence of functional changes without morphologic abnormality in neutrophils has also been suggested by observations on leukocytes removed from patients with diabetes and anemia. Recently, Martin, McKinney, Green, and Becker (9) have reported that leukocytes from diabetics cannot oxidize glucose normally *in vitro* unless insulin is provided. In anemia, Berry and Spies (10) have found that neutrophils, instead of showing disturbed activity, possess greater phagocytic capacities than normal cells.

This state of reduced phagocytic capacity is not a universal feature of the leukemic process, however. In chronic lymphocytic leukemia, in acute granulocytic leukemia, and perhaps in acute lymphocytic leukemias, the individual function of the granulocytes was not usually disturbed. Because of the diminished total numbers of cells, however, the total antibacterial activity of whole blood in these conditions was sometimes less than that of blood from patients with chronic granulocytic leukemia and even less than normal.

It does not seem likely that the decreased activity of neutrophils in granulocytic leukemia was due to irradiation because leukocytes from patients who never received irradiation prior to the study displayed impaired phagocytic activity. Moreover, irradiation appears to enhance rather than depress phagocytic activity. Thus leukocytes from some patients with lymphocytic leukemia receiving Roentgen irradiation displayed greater phagocytosis than normal. In animals, irradiation has also produced greater phagocytosis than normal by neutrophils (11). There is little question that in several patients with granulocytic leukemia, radiation therapy actually produced an improvement in phagocytic activity. The effect of urethane appeared to differ from that of irradiation in the one patient studied because there was a decrease instead of increase in phagocytic activity.

Although the function of the individual cell is impaired in chronic granulocytic leukemia, the excess number of granulocytes raises the total antibacterial defense of the whole blood to normal. Since tissues receive their granulocytes from the blood, it is not surprising that the cellular morphology of the inflammatory reaction in infected tissues is normal in granulocytic leukemia. Jaffé (12) found that in these patients the cells comprising the inflammatory reaction had the appear-

ance of normal neutrophils and they were present in normal numbers or greater.

Bacterial infections were not frequent among the patients in this report (Table VII). Only two patients with chronic granulocytic leukemia suffered from infection. These were both mild furuncles which were promptly localized. Three patients with acute granulocytic leukemia appeared to suffer bacterial infections. Two patients had a sore throat from which coagulase positive staphylococci were cultured. These bacteria are frequently present without producing infection and cannot be definitely regarded as the cause of the sore throats. A third patient experienced a generalized pustular eruption. All three cases recovered after receiving antibiotics. No bacterial infections were noted in 12 patients with lymphocytic leukemia. The relatively low incidence of severe infection in all patients studied may be correlated with observations on leukocyte function. In chronic granulocytic leukemia, an excess number of neutrophils offsets poor individual function. In lymphocytic leukemia, and in the acute leukemias, there appear to be enough normally active phagocytes for defense against bacterial infection even though the total numbers may be somewhat lower than normal. Thus, Jaffé (12) observed that patients with very low leukocyte counts could mobilize adequate numbers of neutrophils from isolated foci in the bone marrow to provide good cellular defense reactions to infection in the tissues. Other cells than granulocytes may also contribute significantly to cellular defense in leukemia. An example of this is provided by the patient with acute monocytic leukemia whose promonocytes were so actively phagocytic that whole blood containing them but no other phagocytes possessed total antibacterial properties equal to that of normal blood. The extreme susceptibility of certain patients with acute leukemia to bacterial infections appears to be a function of a marked decrease in total number of mature granulocytes rather than a decrease in function of individual cells.

The antiphagocytic and leukocyte-destroying properties of stored leukemic plasmas are recorded here as a preliminary observation of interest. The two plasmas most destructive for neutrophils were obtained from patients with granulocytic leukemia when their leukocyte counts were low. Yet the activity of their own leukocytes against staphylococci was good in the fresh whole blood from which the stored plasmas were obtained. In one of these patients, however, the total leukocyte count had fallen from 220,000 to 16,500 just before the plasma was obtained. It remains to be determined if the factor in leukemic plasma which is toxic for leukocytes is related to the leukotoxic substance demonstrated by Doan (13), and what role, if any, it may have in the pathogenesis of leukemia.

SUMMARY AND CONCLUSIONS

Phagocytic activity of leukocytes in 45 cases of leukemia was studied by two methods: 1) Suspensions of leukemic and suspensions of normal leukocytes, containing approximately equal numbers of mature granulocytes were inoculated with opsonized type II pneumococcus and the phagocytic activity of leukemic cells was compared to that of normal granulocytes; 2) Heparinized leukemic blood was divided into two portions and buffy coat removed from one to lower the count of mature neutrophils to normal levels. Each portion was inoculated with pathogenic staphylococci and the number of culturable staphylococci were counted before and after 6 hours incubation.

Pneumococci were phagocytosed less actively by mature granulocytes from chronic granulocytic leukemia than by normal granulocytes. In lymphocytic leukemia, phagocytosis of pneumococci by granulocytes was usually normal. Treatment of patients by irradiation sometimes restored normal phagocytosis in granulocytic leukemia.

Similarly, in chronic granulocytic leukemia, when buffy coat was removed to leave a count of 5,000 to 10,000 mature granulocytes per ml. of blood, the number of culturable staphylococci was often not significantly lowered. Yet normal bloods averaging 5,000 mature granulocytes markedly reduced the numbers of staphylococci. If buffy coat was not removed, blood from chronic granulocytic leukemia also reduced markedly the number of staphylococci.

In chronic granulocytic leukemia, phagocytosis by individual granulocytes was usually impaired but overproduction of granulocytes raised the total defense of the blood to normal. In chronic lymphocytic, and certain acute leukemias, individual function of mature granulocytes is usually undisturbed, but decrease in total numbers sometimes weakens antibacterial defense.

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STUDIES ON HYDRALAZINE; EVIDENCE FOR A PERIPHERAL SITE OF ACTION [1]

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Since its introduction in 1951, hydralazine (1-hydrazinophthalazine, Apresoline®) has become a widely used agent for the treatment of hypertensive disease. The mechanism by which it exerts its hypotensive action has not, however, been clearly defined. This is particularly true for the dosage range which is clinically the most relevant, namely, the smallest amount of the drug which will produce a definite hypotensive effect in patients.

According to the most widely accepted theory, the influence of hydralazine is exerted centrally, perhaps in the mid-brain, where it acts to suppress the outflow of sympathetic vasopressor impulses (1–5). It has also been suggested that the drug functions as a ganglionic blocking agent (2, 6) and that it acts to produce adrenergic blockade (3, 7, 8). One investigator (9) has reported the inhibition of a cerebral vasopressor substance by hydralazine. Except for the implication of this latter report, little consideration has been given to the possibility that the drug may act directly upon the blood vessel wall. The following limited series of observations suggest that hydralazine can act directly in this manner to decrease peripheral resistance.

METHODS AND MATERIALS

Subjects for the blood flow studies consisted of three groups: 1) three patients with uncomplicated hypertensive disease and one hypertensive patient who had largely recovered from a right hemiplegia of two years' duration; 2) four patients with transection of the spinal cord. Complete denervation of the legs was demonstrated by motor and sensory loss below the transection together with an absence of skin temperature response to the 20.5° C. of the constant temperature room; 3) one hypertensive patient who had undergone unilateral lumbodorsal sympathectomy four years before. Completeness of autonomic denervation was again demonstrated by absence of skin temperature response to a cold stimulus. Studies were performed with subjects in the fasting state and, except for one patient who required 50 mg. of Demerol for the relief of pain, none had received any medication the day of the experiment or had smoked for 30 minutes before tests were begun.

Skin temperature and blood flow measurements were carried out in a constant temperature room maintained at 20.5 (± 1) degrees Centigrade with relative humidity between 51 per cent and 57 per cent and wind velocity less than 1 meter per second. The experimental period was of at least three hours' duration, allowing a minimum of two hours' adaptation to the room temperature. Skin temperatures were recorded automatically from the plantar surface of each great toe, the outer surface of each calf and the palmar surface of each middle finger by means of a six point Leeds and Northrup Speedomax which registered the temperature of each point once every 22.5 seconds. Blood flow to forearms and legs was measured by venous occlusion plethysmography using apparatus previously described (10).

Infusion of norepinephrine (Levophed®) before and after administration of hydralazine [3] was performed in two normotensive patients without evidence of significant organic disease. A *Y* tube was connected to the infusion needle to allow rapid and easy change from and to the control infusion of isotonic saline. Blood pressure was measured every one to five minutes on the opposite arm by the auscultatory method and mercury sphygmomanometer, and pulse rate was determined at the same time.

Cold pressor tests were performed 23 times on 13 hypertensive subjects before and after intravenous injection of hydralazine. Throughout the test the patients lay quietly on their own beds in a relaxed state, often falling asleep. The hand was immersed in ice water for a period of one minute as described by Hines (11), except for one patient who could not tolerate more than 30 seconds' immersion;

[1] Supported in part by grants from the Albert and Mary Lasker Foundation, the National Institutes of Health, U. S. Public Health Service and the Public Health Research Institute of the City of New York.

[2] Present address: Department of Medicine, Cornell University Medical College, New York, N. Y.

[3] Hydralazine (1-hydrazinophthalazine, Apresoline®) was generously supplied by Dr. E. A. Reilly of Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

TABLE I

Effect of hydralazine on skin temperature and blood flow of the extremities

Name	Dose of hydralazine mg.	Blood pressure mm. Hg	Skin temperature (° C)		Blood flow (cc./min./100 cc. tissue)	
			Left middle finger	Left great toe	Arm	Leg
Control						
J. B.	15	210/130–195/115	22.5–21.5	21.5–21.5		2.1–1.1
S. S.	15	210/110–175/ 90	20.5–19.5	22.5–20.5		—
J. M.	15	220/130–200/110	24.0–21.0	22.0–21.0		0.7–0.7
J. R.	15	180/110–155/ 90	24.0–21.0	20.0–20.0		0.9–0.5
Paraplegics						
D. J.	15	120/ 82–120/ 72	19.0–19.0	33.5–35.5		1.8–2.8
H. R.	15	154/ 90–152/ 78	22.5–23.0	32.5–34.5		3.3–7.3
C. B.	15	140/ 95–115/ 70	23.0–22.5	31.5–35.0	1.0–0.8	1.1–1.9
A. R.	18	115/ 90–115/ 80	23.5–23.5	32.0–35.0	1.0–0.8	1.0–2.5
Sympathectomy						
			Intact toe	Denervated toe	Intact leg	Denervated leg
A. M.	10	225/114–200/106	22.0–22.0	34.0–36.0	0.4–0.3	0.6–2.8
					Intact foot	Denervated foot
	10	210/110–195/ 95	23.0–22.5	34.0–36.5	0.8–0.5	2.5–8.4

blood pressure readings were made every half minute with a mercury sphygmomanometer on the other arm.

RESULTS

Effects of hydralazine on blood pressure in patients with spinal cord transection: Intravenous administration of 15 mg. of hydralazine resulted in falls in diastolic blood pressure ranging from 10 to 25 mm. Hg in each of four paraplegic patients (Table I). Such responses are comparable to those of subjects with intact nervous systems, and similar initial blood pressure levels. Furthermore, the greatest fall, from 140/95 to 115/70, was noted in the patient (C. B.) with the highest spinal cord transection (T 1–5). It is worthy of note that the decreases in blood pressure following a constant dose of hydralazine were similar in both the constant temperature room and in the warmer temperature of the ward.

Control measurements of blood flow: Following the intravenous administration of hydralazine in single doses, measurements in four patients with normal innervation of all extremities revealed falls in skin temperature of as much as 3° C. in the fingers and 2° C. in the toes with significant reduction in blood flow to the legs in two of three patients (Table I).

Measurements of blood flow in patients with spinal cord transection: Measurements of skin temperature and blood flow were performed in four patients with spinal cord transections to compare the response to hydralazine in the denervated lower extremity with the innervated upper extremity. Intravenous administration of 15 mg. of hydralazine (about 0.3 mg. per Kg.) was followed by an increase in skin temperature of at least 2° C. in the toes of each patient, with no significant change

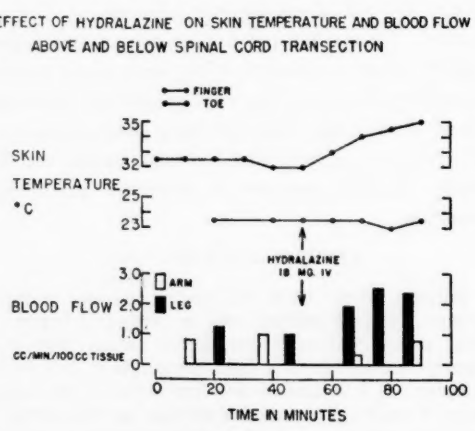

FIGURE 1

Patient A. R.: Two hours in the constant temperature room prior to 0 time on the graph has resulted in adaptation of the intact finger whose temperature is 23.5° C. while the denervated toe remains at 32.5° C. Injection of 18 mg. of hydralazine results in a further rise of 3° C. in the toe, with no change in the finger. Parallel changes are noted in blood flow to the leg and arm.

in the fingers (Table I). Under the conditions of the experiment, changes of 2° C. are considered significant (12). Plethysmography showed significant increases in blood flow to the leg in each patient with slight decrease in blood flow to the arm in the two patients so tested (Figure 1).

Measurements of blood flow in patient with unilateral sympathectomy: In an effort to localize the vasodilator activity of hydralazine distal to the T 9 segment of the spinal cord, studies similar to the foregoing were performed on one patient who had four years previously undergone unilateral lumbodorsal sympathectomy. Here the response of the lower extremity on the sympathectomized side was compared with that of its fellow on the intact side in two experiments, measuring blood flow once through the legs and once through the feet. In the first of these experiments (Figure 2) the toe on the sympathectomized side did not adapt to the 21° C. temperature of the room, but remained at 34° C. Following injection of 10 mg. of hydralazine there was a further rise of 2° C. in the denervated toe, but none in the intact one, with corresponding findings in blood flow to the legs. A fall in blood pressure from 225/114 to 200/106 was recorded in this experiment.

Norepinephrine infusion: Infusion of 2.4 micrograms per minute of norepinephrine in a normotensive patient resulted in a blood pressure rise of 26/18 mm. Hg and a fall in pulse rate of 5 per minute within 10 minutes (Figure 3). After the infusion was discontinued, and the blood pressure and pulse had returned to pre-infusion levels, intravenous administration of hydralazine resulted in a fall in blood pressure of 0/16 mm. Hg with a rise in pulse rate of 20 per min. At this point infusion of norepinephrine at the same rate as before resulted in a rise in blood pressure of 16/22 with a fall in pulse rate of 16 per min. This response occurred from 30 to 50 minutes following injection of hydralazine, at a time when the effect of the drug is maximal. A second normotensive patient responded in the same way.

Cold pressor studies: Cold pressor tests were performed 23 times in 13 hypertensive patients before and after intravenous doses of hydralazine

EFFECT OF HYDRALAZINE ON SKIN TEMPERATURE AND BLOOD FLOW IN INTACT AND SYMPATHECTOMIZED EXTREMITIES

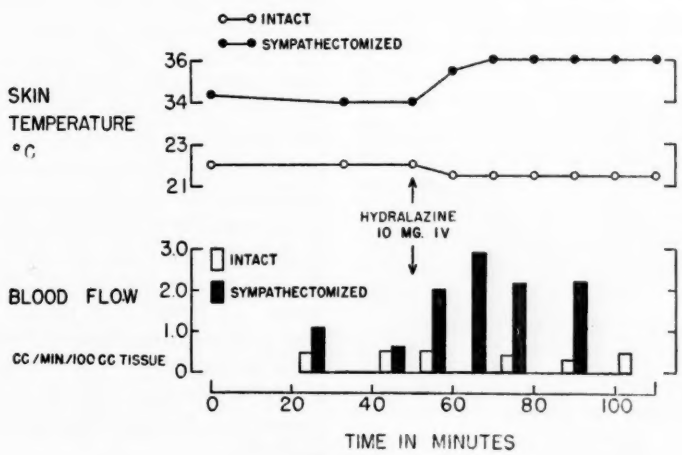

FIGURE 2

Patient A. M.: Adaptation to the constant temperature room has occurred in the intact toe which is 22° C. while the sympathectomized one remains at 34° C. Injection of hydralazine results in a 2° C. rise in the already warmer extremity, with a 0.5° C. fall in the intact one. Parallel changes are noted in blood flow to the two legs.

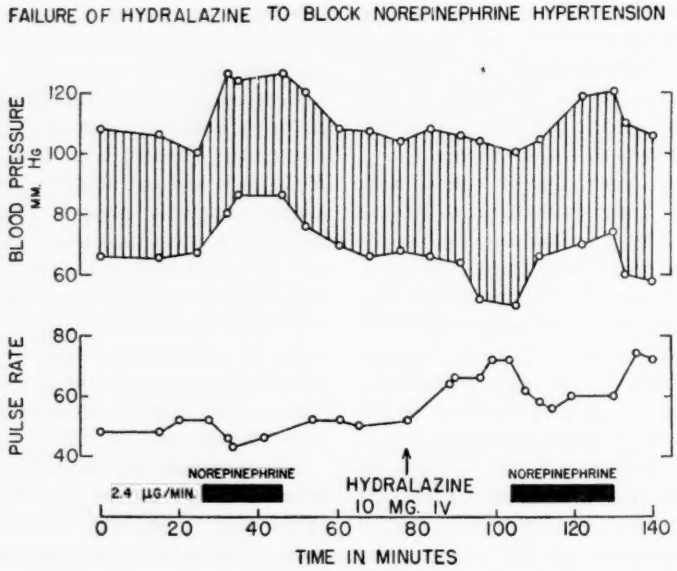

FIGURE 3

Similar rises in blood pressure in response to norepinephrine infusion are found before and after a hypotensive dose of hydralazine. Pulse slowing effect of norepinephrine is not blocked by hydralazine.

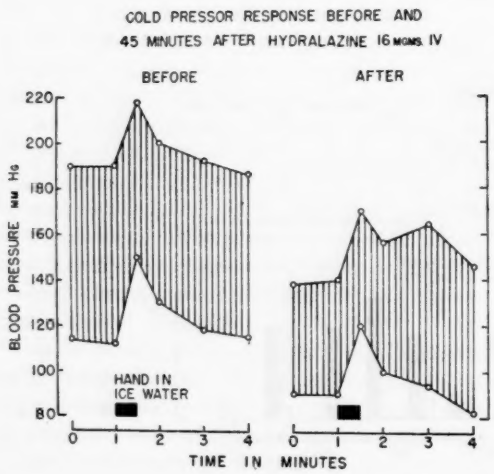

FIGURE 4

A rise in blood pressure of 26/38 is noted in response to immersion of the hand in ice water. Forty-five minutes later, following the administration of 16 mg. of hydralazine with a consequent blood pressure fall of 50/25, the cold pressor response is a rise of 30/30.

ranging from 8 to 22 mg. Of this group 12 tests were performed in eight patients following diastolic blood pressure decreases of at least 10 mm. Hg. During the cold pressor test the average blood pressure rise before hydralazine was 32/31, and after hydralazine 34/29. In no case was there blocking of the response. Eleven tests were performed in eight patients in whom there was no significant fall in diastolic pressure. In this group the average rise during the cold pressor test was 29/28 before the drug and 31/17 afterwards, and the cold pressor test was not blocked in any instance. A typical cold pressor test before and after a hypotensive dose of hydralazine is illustrated in Figure 4. The rise before hydralazine is 26/38, whereas following a fall in diastolic pressure of 25 mm. Hg, the cold pressor rise is 30/30.

DISCUSSION

The theory of the central action of hydralazine is based on the work of Gross, Druey, and Meier (4), and Craver and Yonkman (13) who showed

that the drug does not exert a hypotensive effect in the spinal cat. This is supported by reports (2, 14) that hydralazine blocks vasomotor reflexes.

Denervation studies

Studies comparable to those of Gross, Druey, and Meier, and Craver and Yonkman have not heretofore been performed in man. The results presented here indicate that hydralazine can decrease blood pressure in human subjects with high thoracic spinal cord transections. Furthermore, the drug increases blood flow in extremities denervated either by such transection of the spinal cord or by sympathectomy.

Three explanations of these findings seem possible. First, the increased blood flow may be a result of altered circulatory dynamics, specifically of an increased cardiac output, and thus represent no more than passive adjustment to a greater volume of flow. While hydralazine does increase cardiac output, the increase would appear inadequate to increase blood flow in the denervated extremity in the presence of the decreased blood pressure noted in the present studies.

The second possibility, that these findings result from inhibition of a circulating humoral agent, seems remote in the absence of evidence for such an agent in normotensive subjects.

The third explanation is that hydralazine acts directly upon the blood vessel wall.

The latter hypothesis would account for the otherwise inexplicable finding of Wilkinson, Backman, and Hecht (5) that intra-arterial injection of hydralazine results in a marked increase in skin temperature in the injected extremity, and not in the others, a phenomenon hard to reconcile with a central action of the drug. It does not, however, explain why the vasodilator action is apparent only in denervated limbs, or following intra-arterial injection. There is, of course, the possibility that the action of the drug is a general one on all the blood vessels of the body, but that the greater sympathetic tone under conditions of cold prevents vasodilation from becoming manifest in the normally innervated extremity. This inference may be drawn from the work of Wilkinson, Backman, and Hecht (5) and Grimson, Chittum, and Metcalf (14) who showed an increase in skin temperature following intravenous administration of hydralazine in a warm room. However, the fact that blood pressure response to a constant dose of the drug is similar in warm and cold environments indicates that vasodilatation of skin vessels is a minor effect of hydralazine.

It is unlikely that action on the blood vessel wall in itself is sufficient explanation for the particular pattern of vasodilatation produced by hydralazine. For, if it were true, drugs with a similar site of action might be expected to effect a similar pattern. And nitrites, which are generally believed to act on the arteriolar wall, are reported to produce peripheral vasodilatation and renal vasoconstriction (15), effects opposite to those of hydralazine.

It would appear necessary to postulate a more potent effect of hydralazine on the visceral than on the cutaneous vascular beds. The widely differing effects of the drug on blood flow in the skin and kidney make this a reasonable hypothesis. Furthermore, it focuses attention on the importance of the visceral vasodilator action of hydralazine and the need for study of its mechanism.

Norepinephrine infusion studies

Reports of adrenergic blockade by hydralazine (2, 7, 8, 13, 16) assume a greater significance with the demonstration of its increased vasodilator activity in denervated extremities, for it raises implications of the heightened adrenergic sensitivity of such structures (17, 18). It is, therefore, noteworthy that the doses of hydralazine mentioned in these reports were larger than those found adequate in this study to produce a hypotensive effect. Gross, Druey, and Meier (4), working with cats, has reported that whereas hydralazine in doses of 0.5 mg. per Kg. may decrease the rise in blood pressure following epinephrine (but not norepinephrine), 0.1 mg. per Kg. has no such effect.

Conflicting reports complicate understanding of this problem. Later, more detailed studies (19) in animals have shown that even very large doses of hydralazine (1 to 5 mg. per Kg.) affected pressor responses to epinephrine and norepinephrine "minimally, if at all", "left uninfluenced the response of salivation and the nictitating membrane to epinephrine and norepinephrine" and failed to block their action on the seminal vesicle of the guinea pig. It was concluded that hydralazine's "adreno-

lytic activity would appear to be a secondary characteristic."

The evidence presented above would indicate that doses of hydralazine sufficient to produce a hypotensive effect will not block the hypertensive or pulse-slowing actions of norepinephrine. Such hypotensive activity must, therefore, depend upon a mechanism other than adrenergic blockade.

Cold pressor studies

Demonstration that a drug can produce vasodilatation by peripheral action does not preclude the possibility that it may act also, and even more significantly, through a central neurogenic mechanism. If such were the case, however, hypotensive doses might be expected to inhibit at least the more prominent sympathetic responses such as the vasopressor reflexes. That hydralazine has such an action is frequently cited on the authority of Freis and Finnerty (2) and Grimson, Chittum, and Metcalf (14) who reported suppression of vasomotor reflexes following its administration. It should be noted, however, that the latter authors, using intravenous doses of as much as 20 to 125 mg., observed blocking of the cold pressor test in only one of five patients and found it unchanged in two.

It is clear that large enough doses of any hypotensive agent will ultimately interfere with responsivity to vasopressor stimuli. The demonstration of such effects following very large doses is, therefore, largely irrelevant to the mode of action of a drug in its therapeutic range.

In the present study, hypotensive effects were achieved 13 times with little if any suppression of the cold pressor test. Accordingly, it was concluded that the hypotensive activity of hydralazine in these instances did not depend upon its suppression of those vasopressor stimuli which determine the cold pressor reflex. This observation would also appear to cast doubt upon the theory of central action of hydralazine, and suggests that its major site of action may be the peripheral one previously demonstrated.

SUMMARY

1. Hydralazine in doses of 0.3 mg. per Kg. lowered the blood pressure of four subjects with spinal cord transection as high at T 1–5.

2. Hydralazine increased blood flow in extremities denervated by spinal cord transection and sympathectomy. Such increases were not found in normally innervated extremities.

3. Doses of hydralazine sufficient to lower blood pressure did not interfere with the subsequent hypertension and bradycardia produced by norepinephrine infusion.

4. Hypotensive doses of hydralazine did not interfere with the rise in blood pressure produced by the cold pressor test.

5. It is concluded that hydralazine can act directly upon the blood vessel wall to produce vasodilatation, and that this may be its major site of action.

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THE FIBRINOLYTIC ACTIVITY OF STREPTOKINASE ACTIVATED HUMAN PLASMIN [1]

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The successful introduction of enzymes for the dissolution of undesirable collections of sanguineous, fibrinous, and purulent exudations in localized areas of disease in man (1), has stimulated studies on the experimental *in vivo* dissolution of intravascular blood clots by enzymatic means (2–4). Activation of *plasminogen*, the proteolytic enzyme precursor present in plasma, to *plasmin*, by streptokinase [2] has received considerable attention in these studies (2, 3, 5) not only because of plasminogen's natural occurrence, but because of the special fibrinolytic propensities attributed to SK activated human plasmin (6, 7).

Preparations of the human proenzyme prepared by the Kline procedure (8) and activated by streptokinase, consistently have fibrinolytic activities of approximately 200,000 units per mg. nitrogen.[3] The striking fibrinolytic activity of streptokinase activated human plasmin preparations is very large when compared to their known and measurable proteolytic activity. It seems unusual for an enzyme which extensively attacks a great variety of protein substrates (9–11), and has the same spectrum of activity as trypsin on synthetic substrates (12), to have such a unique fibrinolytic activity.

It is the purpose of this report to present evidence that, (a) the fibrinolytic activity of human plasmin preparations is comparable to that of other known proteolytic enzymes, and (b) the extraordinary fibrinolytic activity of streptokinase activated human plasmin, when measured with the conventional bovine clot, is due to the activation of a contaminating bovine plasminogen in the fibrinogen preparation used in the assay system. This contaminating plasminogen is rapidly activated by streptokinase in the presence of trace amounts of human plasminogen or plasmin. The activation of the bovine plasminogen, under these circumstances, results in abnormally high values for the fibrinolytic activity of human plasmin preparations. In general, the values obtained with the present fibrinolytic assay for streptokinase activated plasmin are approximately five hundred fold greater than the actual fibrinolytic activity of these human plasmin preparations.

The significance of these findings in relation to the diversity of data concerning the streptokinase activated plasma proteolytic system will be discussed.

METHODS

Enzyme preparations:

Human plasminogen. Prepared by the method of Kline (8). This procedure consistently yielded preparations of high activity.

Human plasmin. Three preparations of this enzyme were studied;

a) Human plasminogen activated by streptokinase, and referred to as streptokinase activated plasmin or SK plasmin.

b) A preparation of human plasmin derived from human plasminogen which spontaneously activated while human plasminogen was being reprecipitated three times during a three day period, according to the Kline procedure for plasminogen precipitation.

c) A spontaneously activated human plasmin preparation (Cohn's plasma Fraction III_3), obtained through the courtesy of Dr. D. M. Surgenor, Harvard University Laboratory of Physical Chemistry.

Bovine plasminogen. Bovine plasma Fraction III_2 [4] or fresh plasma were used as sources of bovine plasminogen.

Bovine plasmin (or bovine fibrinolysin). Obtained through the courtesy of Dr. E. Loomis, Parke, Davis and Co. This is a chloroform activated enzyme derived from bovine plasma (13).

[1] This study was supported by a grant from the Lederle Laboratories Division, American Cyanamid Company.

[2] Also referred to as SK.

[3] An activity of this amount is represented by the ability of 0.005 gamma of plasmin N to digest a standard fibrin clot in 30 minutes.

[4] Obtained through the courtesy of Dr. A. H. Holland, Jr. of Armour Laboratories.

Other animal plasminogens. Fresh plasma was used as a source of dog plasminogen. Prothrombin free dried rabbit plasma (Difco) served as a rabbit plasminogen source.

Trypsin and Chymotrypsin (crystalline) (Worthington Labs.).

Streptokinase. A commercial preparation of Varidase (Lederle) (Lot No. 7-1089-287A) containing only small amounts of contaminating inhibitor. This preparation contained a number of other impurities including several non-proteolytic streptococcal enzymes. All references to unitage are in terms of Christensen units (14).

Substrates:

Bovine fibrinogen. Fraction I (Armour), an alcohol fraction of bovine plasma, was used in these studies. A number of different lots of this reagent were employed. Clots made with this preparation plus bovine thrombin [5] in the presence of various amounts of SK, were stable for 24 hours.

Plasminogen-deficient human fibrinogen. A sample of human fibrinogen obtained for study through the courtesy of Cutter Laboratories was further purified by three ammonium sulfate precipitations according to Laki's procedure (15). Although this preparation contained traces of human plasminogen, the contamination was not significant enough to influence the fibrinolytic assay. Clots produced with this reagent plus human [6] or bovine thrombin, in the presence of SK, were stable for several hours. Furthermore, this human fibrinogen preparation was found to be relatively free of both plasmin inhibitor and antistreptokinase, and was suitable for use as a reagent in the fibrinolytic assay.

Casein. A preparation (Hammarsten quality) obtained from Nutritional Biochemicals.

TAMe. (p-toluenesulfonyl l-arginine methyl ester). Synthesized in our laboratories by a modification of the method of Bergman, Fruton, and Pollok (12, 16).

LEe (l-lysine ethyl ester). Synthesized according to conventional methods (17).

Assays:

Fibrinolytic assay for streptokinase activated plasmin, spontaneously activated plasmin, and other proteolytic enzymes. The conventional assay utilizing a clot made with bovine reagents, according to the method of Christensen (14), was employed. When a clot made with human fibrinogen and thrombin was used, the procedure was similar to the Christensen method except for the substitution of human reagents in similar concentrations to the bovine reagents. In the fibrinolytic assay, the enzyme solution to be assayed is serially diluted with buffer, followed by the addition of fibrinogen and thrombin.[7] When streptokinase was used as an activating agent for plasminogen, 1,000 units was incorporated into each of the assay tubes prior to the addition of thrombin. A fibrinolytic unit is defined as the smallest amount of enzyme which produces clot lysis in 30 minutes under the conditions of the test (14).

All reagents in this assay were made up in 0.025 M phosphate buffer in 0.85 per cent sodium chloride at pH 7.5.[8]

Proteolytic assay. According to the casein assay of Remmert and Cohen (10). One casein unit is equivalent to the liberation of 450 gamma of acid soluble tyrosine under the conditions of the test.

TAMe and LEe esterase activity. According to the method previously described (12). The results are expressed as micromoles of acid liberated in 30 minutes at 37° C. under the conditions of the test.

[5] The commercial bovine thrombin preparations of Upjohn and Parke, Davis and Co. were utilized. They contain approximately 25 N.I.H. clotting units per mg. material.

[6] A preparation obtained from Cutter Laboratories containing 20 N.I.H. clotting units per mg. material.

[7] At the present state of purity of the reagents the action of trypsin and chymotrypsin appears to be greater on fibrinogen than fibrin, whereas the reverse is true with SK plasmin.

[8] The presence of phosphate in the clotting mixture markedly accelerates plasmin fibrinolysis. Optimal acceleration was noted with 0.025 M phosphate concentration. The effect of phosphate ion on fibrinolysis is being investigated.

RESULTS

I. *The "unusual" fibrinolytic activity of SK activated human plasmin as compared to other plasmin preparations and trypsin*

In Table I is shown a comparison of crystalline trypsin and several plasmin preparations, against three types of substrates which are attacked by both enzymes, *i.e.*, casein, synthetic basic amino acid esters, and fibrin. The conventional assay of fibrinolytic activity was carried out with a bovine fibrinogen—bovine thrombin clot. Three types of plasmin preparations were assayed: a) an SK activated preparation, b) two spontaneously activated preparations, and c) a chloroform activated bovine plasmin.

From the data in Table I it will be noted that per milligram of nitrogen, trypsin, when compared to the most purified human plasmin preparations presently available, had 30 times as much proteolytic activity against casein, 140 fold the TAMe esterase activity, and 10 times the LEe esterase activity. However, the streptokinase activated human plasmin had a fibrinolytic activity 12 fold greater than trypsin, resulting in a fibrino-

TABLE I

Comparison of the proteolytic, amino acid esterase, and fibrinolytic activity (bovine reagents) of several plasmin preparations, and trypsin

Enzyme	Casein units per *mg. N*	Esterase activity *μM hyd./30 min./mg. N* TAMe*	Esterase activity *μM hyd./30 min./mg. N* LEe†	Fibrinolytic assay units per mg. N (Bovine clot)	Fibrinolytic units per casein unit
Trypsin	2,015	107,000	5,000	14,900	7.4
Plasmin, human (Streptokinase activ. human plasminogen)	63	748	551	176,000	2,790
Plasmin, human (Spont. activated)	58	658	450	438	7.6
Plasmin, human Fr. III_3 (Spont. activated)	4.3	—	—	32	7.4
Plasmin, bovine	1.0	25	10	8	8.0

* p-toluenesulfonyl l-arginine methyl ester.
† l-lysine ethyl ester.

lytic/proteolytic ratio 400 times greater than trypsin. This implies that per unit of proteolytic activity against casein, the streptokinase activated enzyme had a fibrinolytic activity 400 times that of trypsin. These differences in fibrinolytic activity, per proteolytic unit, entirely disappeared when streptokinase was not used as the activating agent. The spontaneously activated human enzymes, and the chloroform activated bovine plasmin had the same fibrinolytic activity per unit of proteolytic activity as trypsin.

II. *Lack of "unusual" fibrinolytic activity of SK activated human plasmin when measured with a human clot*

The striking fibrinolytic activity of streptokinase activated human plasmin also disappeared when the fibrinolytic activity was assayed against a human fibrinogen—human thrombin clot rather than the usual bovine clot.

In Table II the fibrinolytic activity of crystalline trypsin, crystalline chymotrypsin, and several plasmin preparations as determined by assay with a clot made of human reagents, is compared to their proteolytic activity.

The data in Table II reveal that the streptokinase activated human plasmin, which had assayed at 176,000 fibrinolytic units/mg. N. with the bovine reagents, had only 480 fibrinolytic units/mg. N. when assayed against a standard clot composed of human reagents. Furthermore, the fibrinolytic activity per unit of proteolytic activity for the streptokinase activated plasmin was now closely comparable to that observed with the other plasmin preparations, trypsin and chymotrypsin.

The striking fibrinolytic activity of the SK activated human plasmin, as measured by the bovine clot assay, does not appear to be an accurate measure of the actual fibrinolytic activity of the SK plasmin preparation, and can be shown to be due to two phenomena: 1) streptokinase, in the presence of trace amounts of *human* plasminogen or plasmin, is an excellent activator for bovine and other animal plasminogens, and 2) bovine fibrinogen in the amounts used in the fibrinolytic assay is seriously contaminated with bovine plasminogen.

III. *Activation of animal plasminogens by streptokinase in the presence of human plasminogen or plasmin*

Figure 1 illustrates that streptokinase, in the presence of trace amounts of human plasminogen,

TABLE II

Comparison of the proteolytic and fibrinolytic activity (human reagents) of several plasmin preparations, trypsin, and chymotrypsin

Enzyme	Casein units per *mg. N*	Fibrinolytic assay (human clot) (*units per mg. N*)	Fibrinolytic units per casein unit
Plasmin, human* (SK activated human plasminogen)	63	480	7.6
Plasmin, human (Fraction III_3) (Spont. activated)	4.3	30	7.0
Plasmin, bovine	1.0	7.5	7.5
Trypsin	2,015	12,800	6.3
Chymotrypsin	1,177	2,870	2.4

* This preparation contained 176,000 fibrinolytic units per mg. N when assayed with bovine reagents.

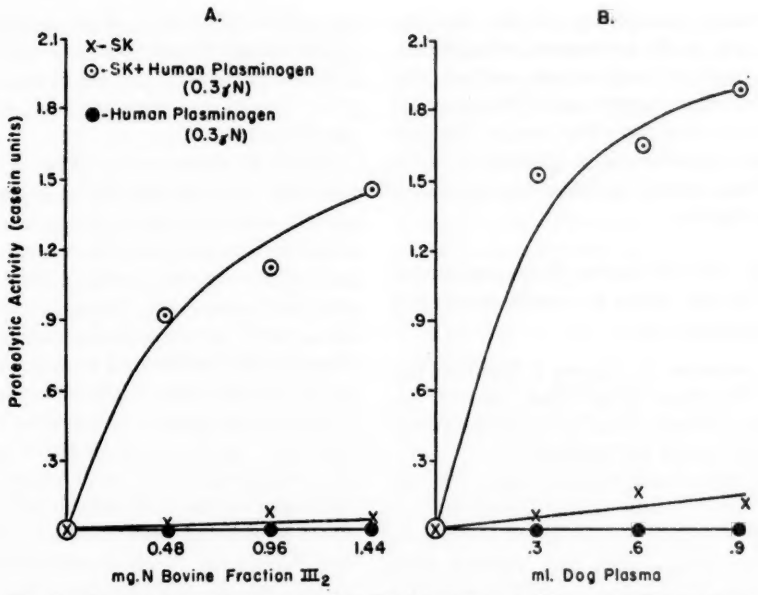

FIG. 1. ACTIVATION OF BOVINE AND DOG PLASMINOGENS BY SK IN THE PRESENCE OF HUMAN PLASMINOGEN

Proteolytic activity formed measured by casein assay (ordinate). When SK was used, 1,000 units was added to test system.

A. BOVINE SYSTEM

Abscissa plots concentration of bovine fraction III_2, used as source of bovine plasminogen in test mixture.

B. DOG SYSTEM

Amounts of dog plasma, used as source of dog plasminogen, in test mixture is shown along abscissa.

is an excellent activator for both bovine and dog plasminogen, yet is without significant effect in the absence of the human factor.

For these experiments, bovine Fraction III_2 was used as a source of bovine plasminogen (Figure 1A) while dog plasma was used as source of dog plasminogen (Figure 1B). Plasmin activity was measured by casein proteolysis. The human plasminogen employed was the highly purified preparation referred to in Table I as having 63 casein units per mg. N following SK activation. Note that only *0.3 gamma* N of this human plasminogen preparation was necessary to show this effect.

Similar observations on the activation of animal plasminogens have been made on: a) bovine plasma as the source of bovine plasminogen; b) dried rabbit plasma as the source of rabbit plasminogen; and c) *in vivo* in the dog following the intravenous injection of small amounts of human plasminogen mixed with SK (3).

The addition of bovine, dog or rabbit plasma, or of bovine Fraction III_2 to a mixture of streptokinase and trace amounts of human plasmin, markedly accelerated the fibrinolysis of a standard human or bovine fibrinogen-thrombin clot.

The activation of these animal plasminogens by streptokinase was observed to occur either in the presence of *human plasminogen or human plasmin.* However, the presence of human plasminogen, or of human plasmin, alone, was without effect as an activator. The possible mechanism of this activation will be subsequently referred to in the discussion. It is probable that the human factor required in this activation is plasmin, or perhaps plasminogen, rather than some contaminant of the plasminogen preparations, since a) purification of human plasminogen or plasmin, of up to 300 fold, by a number of methods (8, 10, 17, 18) has resulted

in a simultaneous purification of this activator function (12), b) in the presence of streptokinase, only minute traces of some of our purified plasminogen preparations (as little as 0.005 *gamma* N) are necessary to show this effect, and c) inactivation of human plasminogen or plasmin by a variety of methods, results in a simultaneous loss of the activator function.

IV. *Evidence that the bovine fibrinogen used in the fibrinolytic assay is contaminated with bovine plasminogen*

The data presented in Figure 2 illustrate that the bovine fibrinogen preparation usually employed in the fibrinolytic assay is seriously contaminated with bovine plasminogen.

In the experiment described in Figure 2A., the digestion of casein, by the bovine fibrinogen used in the fibrinolytic assay, was measured in the presence of streptokinase with and without trace amounts of human plasminogen (0.3 *gamma* N). It will be seen that significant proteolysis was observed in the presence of the mixture of streptokinase plus trace amounts of human plasminogen, but not in the presence of plasminogen or streptokinase alone. These results indicate the activation of a proteolytic enzyme in the bovine fibrinogen preparation by the mixture of SK plus human plasminogen.

Figure 2B illustrates the effect of bovine fibrinogen, due to its bovine plasminogen contaminant, on fibrinolysis of a human clot. For this experiment, various amounts of bovine fibrinogen in 0.5 ml. buffer were added to 0.5 ml. of 0.5 per cent human fibrinogen. One thousand units of streptokinase in 0.1 ml. were then added and the mixture clotted by the addition of 1 unit of human thrombin in 0.1 ml. buffer. The lysis time of the clots were then measured. The data in Figure 2B. reveal that significant fibrinolysis appeared with the incorporation of small amounts of bovine fibrinogen in the test system, and was progressively accelerated with increasing amounts of bovine fibrinogen. With the test system employed, a concentration of 0.25 per cent bovine fibrinogen, the amount used in the routine fibrinolytic assay, produced clot lysis in 12 minutes.

These observations have been confirmed on all

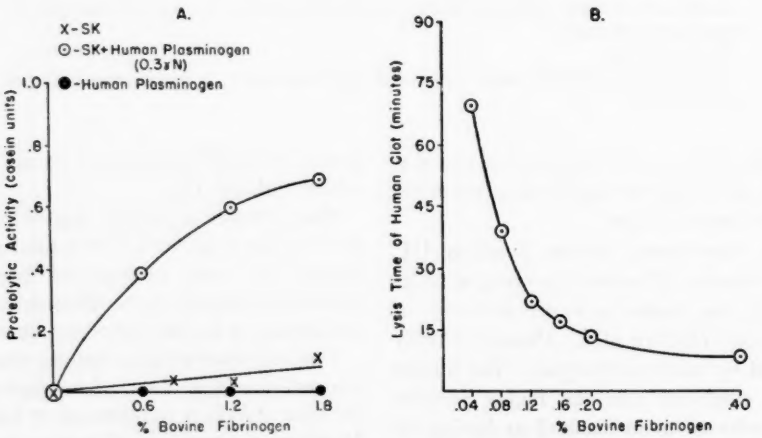

FIG. 2. A. ACTIVATION OF A PROTEOLYTIC ENZYME IN BOVINE FIBRINOGEN REAGENT BY STREPTOKINASE IN THE PRESENCE OF HUMAN PLASMINOGEN

Proteolytic activity found was measured by casein assay (ordinate). Abscissa plots concentration of bovine fibrinogen in test mixture. When SK used, 1,000 units was added to test system.

B. ACCELERATION OF FIBRINOLYSIS OF A HUMAN CLOT BY THE ADDITION OF BOVINE FIBRINOGEN TO THE CLOTTING MIXTURE

The ordinate plots the lysis time of the human clot in the presence of SK, and the abscissa the amount of bovine fibrinogen added to the clotting mixture. See text for details.

of the lots of bovine fibrinogen tested. Although each lot differed somewhat quantitatively in the amount of bovine plasminogen present, they were qualitatively similar.

That these findings are attributable to a contamination of the fibrinogen with bovine plasminogen rather than associated with bovine fibrinogen itself was demonstrated, by studying the digestion of casein, or the acceleration of fibrinolysis, with the fluid expressed after the bovine fibrinogen was clotted with thrombin. Although some of the bovine plasminogen was adsorbed on the clot, sufficient amounts were present in the supernatant to confirm these observations. A number of attempts have been made to fractionate and concentrate the bovine plasminogen in the fibrinogen, by the procedure used for human plasminogen. The bovine plasminogen, however, appears to be much more labile, readily denaturing at acid pH's. We are pursuing this phase of the investigation, however, in order to determine whether the bovine plasminogen has similar characteristics to the human enzyme.

Further evidence for the contamination of bovine fibrinogen with bovine plasminogen was obtained by purification of the bovine fibrinogen. When more highly purified bovine fibrinogen preparations were used as substrates in the fibrinolytic assay, there was a sharp reduction in the measurable fibrinolytic activity of streptokinase activated human plasmin. For this experiment two samples of bovine fibrinogen (Armours Fraction I, and a sample of fibrinogen obtained through the courtesy of Dr. Walter Seegers) were further purified by ammonium sulfate fractionation according to the technique described by Laki (15). A purified human plasminogen preparation[9] was assayed for its streptokinase activatable fibrinolytic activity utilizing each of the four different fibrinogens at the same clottable protein concentration (0.15 per cent fibrinogen in the final clot). The fibrinolytic assay of the SK activated human plasmin with the original Armours Fraction I was 175,000 units per mg. N. With the purified Fraction I, the assay fell to 4,400 units per mg. N, or one-fortieth of the original assay. With the original Seegers fibrinogen, the SK activated human plasmin specimen assayed at 350,000 units per mg. N. After purification of this fibrinogen, the SK plasmin assayed at only 820 units per mg. N, or approximately one four-hundredth the original assay.

Plasminogen-deficient bovine fibrinogen could be readily prepared by subjecting the Armours Fraction I to a three-fold repurification according to the Laki technique (15). When this preparation was used as a substrate in the fibrinolytic assay, the assay of SK activated human plasmin was similar to that found with purified human fibrinogen.

The bovine thrombin preparations used in the fibrinolytic assay can also be readily demonstrated to contain bovine plasminogen. However, in the amounts used in the assay (1 thrombin unit) insignificant amounts of the contaminating plasminogen are added, and do not affect the assay. However, if ten or more units of thrombin are added to the clot, a significant effect on the assay becomes apparent.

V. *The influence of the bovine plasminogen contaminant on the fibrinolytic assay for streptokinase activated plasmin*

It should be re-emphasized that the fibrinolytic assay for streptokinase activated plasmin is carried out by serially diluting the human proenzyme preparation and then adding an equal amount of bovine fibrinogen, SK and bovine thrombin to each of the assay tubes. Since the rapid activation of the bovine plasminogen contaminant of bovine fibrinogen requires only traces of human plasmin in the presence of SK, one would predict that while the addition of SK to a fully activated human plasmin preparation would not enhance the proteolytic activity, it should strikingly enhance the conventional fibrinolytic assay. In order to test this hypothesis, the spontaneously active human plasmin preparation, Fraction III_3, was studied in the presence and absence of SK. This preparation had a spontaneous proteolytic activity of 4.3 casein units per mg. N, which did not increase upon the addition of 1,000 units of streptokinase to each ml. of the enzyme preparation. However, the fibrinolytic assay of this preparation before the addition of SK was *32* units per mg. N, and was increased to 16,000 units per mg. N following the ad-

[9] This preparation, following SK activation, had 60 casein units per mg. N.

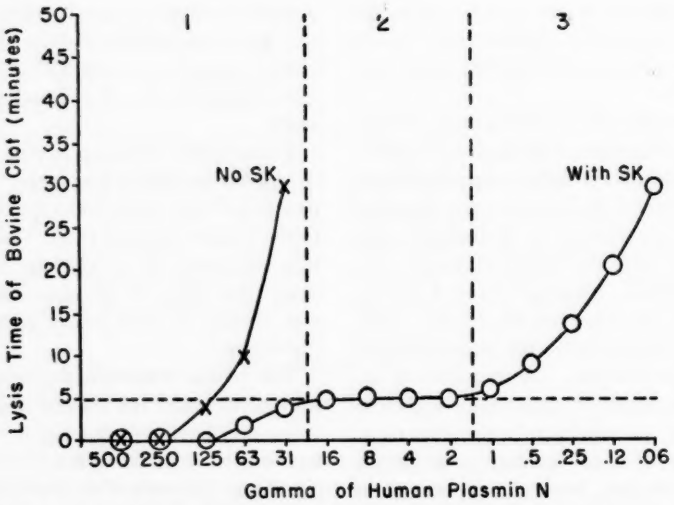

FIG. 3. LYSIS TIME OF BOVINE CLOT AS A FUNCTION OF HUMAN PLASMIN CONCENTRATION (IN THE PRESENCE AND ABSENCE OF SK)

Note that the abscissa plots successive serial dilutions of human plasmin N. See text for details.

dition of SK. The lysis times of the bovine clots with each serial dilution of the human plasmin preparation, in the presence and absence of SK, is shown in Figure 3.

Observations on the lysis time of the bovine clot, by the human plasmin preparation alone, revealed the expected relation of fibrinolysis time to enzyme concentration as seen with other proteolytic enzymes. Upon the addition of SK however, the pattern of fibrinolysis time to enzyme concentration was distinctly different. There was an initial zone where the lysis times were very rapid, but related to enzyme concentration; followed by a second zone where the lysis time was constant even though the human plasmin concentration was reduced 16-fold; followed by a third and final zone where concentration was again related to lysis time.

The kinetics of these observations on clot lysis may be explained by the presence of the contaminating bovine plasminogen in constant amounts in all the assay tubes, and its activation by the human plasmin plus SK. The horizontal broken line in Figure 3 may be interpreted as representing the lysis time of the clot which would result from the maximal activation of the contaminating bovine plasminogen present. The first zone represents the fibrinolytic activity of the activated bovine plasmin plus significant amounts of proteolytic activity derived from the human plasmin. In zone two, the amounts of human plasmin present do not significantly affect fibrinolysis, but are adequate, in the presence of SK, for complete activation of the bovine plasminogen. In zone three, the concentration of human plasmin has finally become a limiting factor in the activation of bovine plasminogen, resulting in a progressive prolongation of the clot lysis time as a function of human plasmin concentration.

Note that the activating function of human plasmin in the presence of SK, on bovine plasminogen, in this experiment, is 500 times the actual fibrinolytic activity of the human plasmin.

VI. *Streptokinase as an essential component of the activating system*

Although not shown in Figure 3, and as would be expected, the lysis time of *human clots*, by various concentrations of the spontaneously activated human plasmin preparation Fraction III_s, was not effected by the addition of 1,000 units of SK, and was similar to that observed for the bovine clots in the absence of SK. These latter observations suggest that human plasmin *per se* does not rapidly activate bovine plasminogen, and that SK

is an essential component of the activating system. This was more effectively demonstrated by studying a preparation of purified human plasminogen which had ben activated to plasmin by SK; then inactivating the SK without destroying the plasmin; and finally adding fresh SK to restore that lost by the inactivating procedure. The inactivation of SK in the presence of purified human plasmin was accomplished by heating the preparation in a boiling water bath for 15 minutes at pH 2.0. Preliminary observations had shown that plasmin was stable at pH 2.0 under these condition,[10] whereas SK, in the presence of human plasmin, was fairly rapidly inactivated by this procedure.

A human plasminogen preparation containing 0.15 mg. N per ml., and to which 17,000 units of SK had been added, was allowed to activate at room temperature for 10 minutes. Assay of the plasmin formed revealed 90,000 fibrinolytic units (bovine clot) and 28.9 casein units per mg. N. Following 15 minutes heating at pH 2.0 in a boiling bath, the proteolytic assay of this preparation, when subsequently adjusted to the original pH, was not significantly altered, but the fibrinolytic activity had fallen to 1,000 units per mg. N. Readdition of 17,000 units of fresh SK restored the fibrinolytic assay to its original 90,000 units per mg. N. Since the fibrinolytic assay, under the conditions of the experiment cited, was primarily a measure of the activating function of the preparation studied, SK appears to be a necessary component of this activating system.

DISCUSSION

On the basis of the observations cited in this report, it appears that the fibrinolytic activity of human plasmin is comparable to that which has been demonstrated for a number of other proteolytic enzymes. In plasma, plasmin appears to attack fibrin more readily than fibrinogen but this may be due, as Ratnoff has suggested, to its ability to activate more readily in the presence of fibrin (19) or to local factors existing on a plasma clot. The data presented indicate that the fibrinolytic activity of plasmin, in a more purified system, is predictable on the basis of its general proteolytic properties. That the action of this enzyme in plasma is not restricted to fibrinogen and fibrin is evident from the recent studies of Pillemer, Ratnoff, Blum, and Lepow on complement destruction (20), and of Ferguson, on the disappearance of Ac globulin (21).

It has recently been suggested that the intravenous injection of purified human plasminogen, which has been activated *in vitro* by SK, may prove useful as a clot dissolving agent in man (5). The basis for this view was the observation of significant circulating proteolytic and fibrinolytic activity following the injection of SK activated human plasmin into dogs. These observations however, have not taken into account, that this type of mixture activates the dogs plasminogen *in vivo*. We have used this procedure to activate the animals own plasminogen *in vivo*, and to evaluate plasmin as a clot dissolver (3). The injection of previously activated purified human plasminogen for clot dissolution in man, although deserving trial, may not be as promising as suspected, because of the low order of activity of the present preparations, as well as the obvious limitations on the amount of material available. However, since plasminogen is the naturally occurring proteolytic enzyme precursor of plasma, it would still appear that the *in vivo* activation of this enzyme in man, by the introduction of SK, may be the most desirable method for directly effecting the enzymatic dissolution of thrombi and emboli.

The observations described in this article reveal that the present assay using bovine fibrinogen and thrombin for the fibrinolytic activity of streptokinase activated human plasmin (14) is not a measure of the actual fibrinolytic activity of plasmin. This assay measures the activator function of human plasmin rather than its fibrinolytic activity. The activator function in the presence of SK can be measured with approximately 1/500 of the amount of enzyme actually required to lyse a clot in thirty minutes. The measurement of the actual fibrinolytic activity of SK activated human plasmin requires more purified fibrinogen than those commercially available.

Similarly the present assay for streptokinase should be re-evaluated. The unit of streptokinase is defined as the smallest amount of SK, which in the presence of human plasminogen will produce lysis of a bovine clot in 10 minutes or less, and was conceived as representing the amount of

[10] True for purified plasmin preparations only, and not for plasma.

kinase needed to rapidly activate sufficient human plasminogen to lyse a clot in the period noted (14). Our data suggest that the unit of SK may represent the amount of SK required in the presence of human plasminogen to rapidly activate the major portion of the bovine plasminogen contaminant present in the bovine reagents.

The observations reported on in this article are of some interest in relation to the recent literature concerning a) the mechanism of activation of plasminogen by streptokinase, b) the presence of a proactivator system in blood for plasminogen activation, and c) the participation of a complement-like factor in the streptokinase activation of human plasminogen:

Recently, the original studies of Christensen and MacLoed (22) on the kinase nature of activation of plasminogen by SK, have been seriously questioned (23). Wasserman, *employing the fibrinolytic assay,* showed that the kinetics of the activation of plasminogen by SK, is compatible with the interpretation of a stoichiometric reaction, and that the formation of plasmin does not conform to the kinetics of an enzymatic reaction. The amount of plasmin formed, in the presence of an excess of plasminogen, was found to be dependent on the SK concentration, and that at all levels of streptokinase, maximal plasmin formation occurred almost instantaneously and did not increase with prolonged incubation.

Although we have been able to confirm Wasserman's observations, they may not yield information on the kinetics of human plasminogen activation by SK. In the fibrinolytic assay, these kinetics may be masked by the activation of the bovine plasminogen contaminating the reagents. Studies on the kinetics of plasminogen activation by streptokinase are also hampered by the impurities which are present in the available SK preparations. Preliminary data obtained with plasminogen-deficient fibrinogen are still in agreement with the initial observations of Wasserman, and suggest that the mechanism by which streptokinase activates human plasminogen may require further clarification.

The demonstration of bovine plasminogen activation by SK in the presence of human plasma, by Mullertz and Lassen (24) led him to hypothesize the presence of a proactivator in human plasma which was activated by SK, and that this activator now converted human and all other animal plasminogens into plasmin. We are inclined to identify this activator function of human plasma, in the presence of SK, to human plasminogen or plasmin itself. This viewpoint is based on a) purification data (12); b) the trace quantities of purified human plasminogen or plasmin preparations required; c) plasmin or plasminogen inactivation causing the loss of activator function; and d) inactivation of streptokinase, following its addition to human plasminogen or plasmin, results in an immediate loss of activator function; and is promptly restored by the addition of fresh SK.

The recent suggestion of Geiger that a complement-like factor participates in the activation of human plasminogen (25) was based on the marked increment of proteolytic activity which followed the addition of fresh animal sera to a mixture of SK and heated human globulin preparations. The heating of the human globulin was shown to inactivate most, but not all, of the human plasminogen. The observations cited in this report suggest that Geiger's results may be alternatively explained as a demonstration of the activation of the plasminogen of the various animal sera by the mixture of small amounts of human plasminogen and SK, without invoking the participation of another factor.

The mechanism for the activation of animal plasminogen by streptokinase in the presence of human plasminogen or plasmin is obscure. It can be shown that the presence of animal plasminogens does not enhance the ability of SK to activate human plasminogen. Our working hypothesis is that the plasma inhibitor to the activation of the various animal plasminogens by streptokinase has a much greater affinity for human plasminogen or plasmin than it does for its respective plasminogen. The addition of the human factor results in a release of the animal plasminogen for activation by streptokinase. An alternative view, also under exploration, is that the rapid activation of plasminogen is carried out by a SK-plasmin complex, and that human plasmin combines more readily with SK in this respect than the plasmin of other animals.

SUMMARY

1. The ratio of fibrinolytic to proteolytic activity of human plasmin, in more purified systems,

is of the same order as that shown by trypsin and chymotrypsin.

2. Streptokinase, in the presence of minute amounts of human plasminogen or human plasmin, activates the bovine plasminogen contaminating the bovine fibrinogen in the fibrinolytic assay.

3. The fibrinolytic assay for streptokinase activated human plasmin, as presently employed with bovine fibrinogen and thrombin, measures the activator function of the human enzyme rather than the fibrinolytic activity of the human plasmin.

ACKNOWLEDGMENT

The author acknowledges with gratitude the many excellent suggestions of Dr. Walter Troll, and the technical assistance of Miss Mary E. Schmidt in this study.

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THE MECHANISM OF FORMATION OF OSMOTICALLY CONCENTRATED URINE DURING THE ANTIDIURETIC STATE[1]

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The present study is concerned with the operation by which the kidney, during antidiuresis, conserves water for the body by elaborating a urine osmotically more concentrated than the blood.

It is appropriate to emphasize that in the mammals the osmotic pressure of the plasma and the interstitial fluid is one of the most closely guarded of all the homeostatic states, and that the kidney is chiefly responsible for the regulation of this osmotic pressure within narrow limits. Osmotic dilution of the plasma is normally offset by the excretion of a urine osmotically more dilute than the plasma, as in water diuresis, while an increase in osmotic concentration of the plasma is offset by the excretion of urine osmotically more concentrated than the plasma. The present paper is concerned only with the mechanism of the excretion of a hypertonic urine.

That no osmotic concentration is achieved in the separation of the glomerular filtrate is established, to the satisfaction of all investigators, by the well known micropuncture studies of Richards and his coworkers in the Amphibia, and Walker, Oliver, and their coworkers in the guinea pig, rat and opossum. These studies have also demonstrated, at least under the recorded experimental conditions, that the urine remains isosmotic with the plasma, or nearly so, throughout the length of the proximal segment, at a time when the bladder urine may be concentrated osmotically to a considerable extent. Unfortunately, such micropuncture studies as are available have failed to explore the question of just where, in the more distal portion of the nephron, osmotic concentration is effected, whether in the distal segment (which can be differentiated cytologically into at least two portions), or in the collecting ducts, or both. We are here not primarily concerned with this anatomical question, but rather with the quantitative evaluation of the overall osmotic operation.

In the process of osmotic regulation as observed during antidiuresis (*i.e.*, in the hydropenic state or during the administration of antidiuretic hormone) the kidney separates from the plasma more osmotically active material than water, and thus produces a urine that is osmotically more concentrated than the plasma. The volume of plasma cleared of osmotically active material is calculated, as with any clearance, as the rate of excretion of osmols per minute, $U_{osm}V$, divided by the osmotic concentration of the plasma, P_{osm}. The osmotic concentrations in urine (U_{osm}) and plasma (P_{osm}) are measured by the reduction of freezing point or, alternatively, the reduction of vapor pressure of urine and plasma, respectively, and V signifies the urine flow in ml. per min. It is convenient to designate this *osmolar clearance*, $U_{osm}V/P_{osm}$, as C_{osm}, which has the same connotation as other familiar clearance expressions and refers to the rate of clearance from the plasma of all osmotically active material, irrespective of its nature.

Reasons will be given later to support the belief that an osmotically concentrated urine is elaborated by the subtraction of water from the isosmotic glomerular filtrate rather than by the addition of solutes. The quantity of water so abstracted per minute has been designated as $T^c_{H_2O}$ by Wesson and Anslow (1) the superfix *c* serving to identify it as the quantity of water abstracted in the concentrating operation.[4] This moiety of water is

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[3] The substance of this paper has been reported in a preliminary way by the senior author (Smith, Federation Proc., 1952, 11, 701; also in *Symposium on renal function in infancy*, M and R Corporation, Buffalo, March 3 and 4, 1953).

[4] This term was originally designated as $T^x_{H_2O}$ by Smith (2) but $T^c_{H_2O}$ is clearly better since it is indicative

given by the osmolar clearance minus the urine flow:

$$(1) \qquad T^c_{H_2O} = C_{osm} - V$$

This study is concerned with the urinary concentrating operation, as represented by equation (*1*), during progressively increasing osmotic diuresis induced by mannitol in hydropenic and hydrated subjects, with and without the simultaneous administration of Pitressin.[5]

METHODS

Our observations have been made on subjects who had been admitted to Bellevue Hospital for minor or elective surgery or were about to be discharged after such surgery. They were selected with care to exclude cardiovascular or renal disease, and ranged in age from 15 to 46 years. Prior to observation they had been on the regular ward diet and unrestricted salt intake.[6] Except where otherwise noted, they had taken no fluids during the previous 12 to 14 hours. In no case were they prehydrated in the sense of Ladd, 8 to 13 hours before the experiment (5), and it is assumed that their physiological state was that of individuals consuming only moderate quantities of fluid up to the time of fluid restriction.

Observations were made in the forenoon while the subjects were in the fasting state. To induce progressive and controllable osmotic diuresis, 10 per cent (hypertonic) mannitol solution was administered by a constant-rate infusion pump at a speed of 20 ml. per min., or a 5 per cent (isotonic) solution was administered at rates up to 40 ml. per min. Urine was collected by an indwelling bladder catheter at intervals of approximately 10 minutes and the bladder emptied with air and manual compression. Blood was drawn through a retention needle from the antecubital vein into heparinized syringes and centrifuged immediately in rubber-capped tubes, and the plasma separated and stored in stoppered tubes.

The freezing-point method of Wesson (6), utilizing a thermistor, was used to measure the osmotic pressure of plasma and urine. To exclude a possible source of error, the osmotic pressure of arterial and venous blood drawn early during the infusion of 10 per cent mannitol at 20 ml. per min. was compared and found to agree within one per cent. The same osmotic pressure was observed when one part of a sample of blood was centrifuged under oil, the other in a capped tube but in contact with air.

The filtration rate was measured by the inulin clearance (C_{IN}). The inulin was administered at a constant rate, after an initial priming dose, by a second infusion pump. The resorcinol method of Roe as modified by Schreiner (7), was used for the determination of inulin in unyeasted urine and cadmium sulfate filtrates of plasma. In the calculation of the inulin and osmotic clearances a delay time of 2.5 minutes was allowed.

Sodium and potassium were determined with a Perkin-Elmer flame photometer (model 52A).

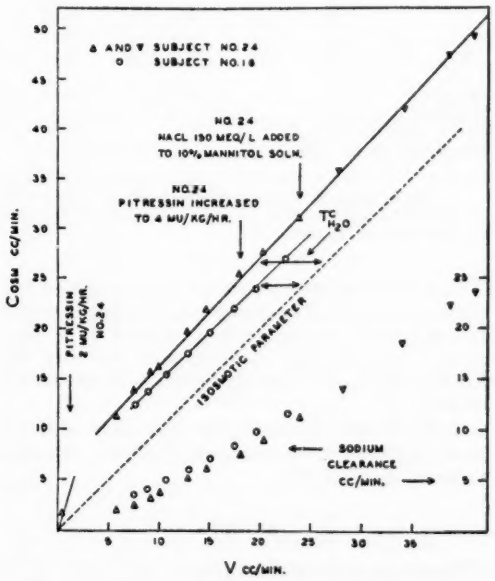

FIG. 1. EXAMINATION OF THE RENAL CONCENTRATING OPERATION IN TWO HYDROPENIC SUBJECTS RECEIVING 10 PER CENT MANNITOL INTRAVENOUSLY

The solute-free water abstracted from the urine is shown by the horizontal difference between the experimental curve and the isosmotic parameter. The slope in No. 24 is 1.063, and in No. 16 is 0.969. In so far as this slope deviates significantly from 1.0, $T^c_{H_2O}$ is not constant, and for the purposes of this paper the value interpolated when V = 20 ml. per min. has been reported in the tables. Possible reasons for inconstancy are discussed in the text.

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of the qualitative nature of the process. Brodsky, Rapaport, and West (3) have called this moiety the water economy of the kidney and indicated it as $H_2O_{E_c}$. Brodsky (4) reports that during hydropenia and mannitol diuresis in man, this term reaches a level of 5 to 7 ml. per min. at urine flows exceeding 4 ml. per min., with small change at urine flows up to about 9 ml. per min.

[5] The present investigation had its origin in Ladd's (5) observation that in subjects who have been prehydrated with a massive dose of water 8 to 13 hours before examination, the infusion of saline coupled with Pitressin leads to copious saline diuresis in which a constant quantity of water (ca. 2.0 ml. per 100 ml. of glomerular filtrate) was abstracted from an isosmotic urine.

[6] Because of the widespread use of antibiotics pre- and postoperatively, it was unavoidable that over half of the subjects had received such medication, usually penicillin. No one, however, had received an antibiotic within 12 hours of study.

TABLE I

Osmotic diuresis (10 per cent mannitol) in hydropenic subjects

1 Subject No.	2 Sex	3 Age	4 S.A.	5 Urine flow range	6 Correl. coeff.	7 Regres. coeff. (slope)	8 $T^{c}_{H_2O}$ per 1.73 sq. m.	9 P_{osm} range	10 P_{Na} range	11 P_K range	12 Mean inulin clearance per 1.73 sq. m.
		yrs.	*sq. m.*	*ml./min.*			*ml./min.*	*mosm/L.*	*mEq./L.*	*mEq./L.*	*ml./min*
1	M	22	1.70	3.6–12.3	.9990	1.168	7.4	287–288	146	5.1–4.9	117
2	M	15	1.60	7.0–20.8	.9992	1.104	4.6	280–289	144	4.4	104
3	M	18	1.73	10.1–21.3	.9998	1.012	5.4	286–295	146–141	4.3–4.8	139
4	F	43	1.72	8.0–23.4	.9997	1.054	4.9	294–300	137	4.7	75
5*	F	18	1.58	21.0–33.4	.9975	1.071	3.4	289–298	140	4.9	130
6	F	34	1.46	9.2–23.4	.9996	1.051	7.2	294–300	144	4.5	108
7	M	37	1.58	8.7–20.5	.9992	.987	4.2	286–295	136	4.9	95
10	M	21	1.49	9.2–23.6	.9998	1.046	6.2	292–293	139–139	4.5–4.9	91
11†	M	46	1.65	6.3–15.8	.9993	1.053	5.3	288–296	138–122	4.2–4.8	76
12	M	27	1.85	11.8–26.2	.9950	.916	4.1	286–291	139–136	4.5–4.4	108
13	F	30	1.57	8.1–26.7	.9999	.928	2.2	285–298	139–125	5.0–6.1	83
14	F	24	1.78	11.4–29.3	.9998	.863	1.5	291–302	135–128	4.6–4.8	85
15	F	27	1.62	9.5–24.2	.9983	1.016	6.4	278–280	134–123	4.1–4.3	137
16	M	18	1.56	7.7–22.7	.9999	.969	4.8	290–303	130–117	4.5–4.9	86
18	M	28	2.00	9.5–19.4	.9989	1.116	6.4	289–291	126–122	4.2–4.5	117
19	M	23	1.98	6.2–17.0	.9992	1.135	6.5	291–294	137–133	4.3–4.5	105
20	M	37	2.11	9.6–17.0	.9982	1.031	5.4	293–296	147–135	4.6–5.2	94
21	F	19	1.32	6.6–19.4	.9998	1.059	6.6	290–301	129–117	4.4–4.7	100
22	F	22	1.60	6.6–15.3	.9961	.976	4.9	286–296	132–118	4.9–5.3	107
23	M	28	1.95	5.5–16.8	.9997	1.02	4.9	287–293	135–132	4.4–4.6	92
27	F	28	1.51	9.3–20.0	.9997	1.029	5.2	291–302	122–116	4.7–6.1	85
Mean				8.8–21.4	.9989	1.033	5.1	288–295	135–127	4.5–4.9	102
Standard deviation						±0.075	±1.5				
Coefficient of variation						0.073	0.29				
Preinfusion mean ± S.D.								283±4.3	141±5.36	4.48±0.27	

* Rapid osmotic diuresis unexpectedly occurred early after the start of the mannitol infusion.

† Found at operation to have carcinoma of the stomach, and longstanding urethral stricture. Sodium excretion was low during the test.

Each series of observations on any one subject consisted of from 5 to 12 or more consecutive urine-collection periods obtained during increasing diuresis. The observations were generally continued until the urine flow had been increased by osmotic diuresis to 30 ml. per min. or more.

In all, 52 series of observations were made on 31 subjects; of these, 2 series on each of 3 subjects were made to check reproducibility.

RESULTS

Hydropenia without Pitressin

Twenty-one studies, on as many subjects (12 males and 9 females), were made postabsorptively and after abstinence from all fluids for 12 to 14 hours. Two to three liters or more of 10 per cent mannitol (20 ml. per min.) were generally required to establish a urine flow of 30 ml. per min. No Pitressin was administered, and no additional water was given during the test except in the form of occasional small pieces of ice to quench thirst. Smoking was not restricted.

Figure 1 shows a series of observations from one subject (No. 16) in this group. The osmolar clearance, C_{osm}, is here plotted against the simultaneous urine flow, V. In interpreting this figure it should be noted that if the glomerular filtrate underwent no osmotic concentration or dilution, the osmolar clearance would be identical with the urine flow over the whole range of observation, *i.e.*, all the data would fall on the bisector, which is here labeled the 'isosmotic parameter' (8). However, the osmolar clearance always exceeds the urine flow, the relations between the two being such that the data generate a linear regression line displaced to one side of the isosmotic parameter by a nearly constant amount, and having a slope of approximately 1.0. This regression line has been designated as the hypertonic parameter, an expression first used by Ladd (5, 8).

There are only two operations by which the kidney can transform the glomerular filtrate from the isosmotic to the hypertonic state: namely, by

the addition to the urine of solute without water, or by the removal of water without solute.[7] During mannitol infusion, the urine flow is increasing primarily because of increasing quantities of mannitol claiming excretion, and the predominant urinary solutes are mannitol and sodium chloride.[8] The concentration of potassium is negligible. The first explanation would require that mannitol or sodium be excreted by the tubules; since it is known that mannitol is excreted solely by filtration, and since there is no evidence for the tubular excretion of sodium, this explanation is unacceptable.

Alternatively, given a variable volume flow of isosmotic urine down the tubules, the rate of flow being determined *inter alia* by the amount of unreabsorbed mannitol, passage from the isosmotic to the hypertonic state could be effected by the reabsorption of water, thus reducing the urine flow below the simultaneous osmolar clearance. This is the interpretation which we propose, and in Figure 1 this quantity of reabsorbed water is designated as $T^c_{H_2O}$.

Table I summarizes the pertinent data on the 21 subjects in this group. Columns 1 to 4 of this table are self-explanatory; column 5 records the range of urine flow involved in the calculations shown in columns 6, 7, and 8; for reasons which will be discussed later, observations at urine flows below a critical value, which varies in different subjects, are excluded from these calculations. Column 6 records the coefficient of correlation calculated for linear regression. Column 7 records the slope of the regression line (regression coefficient). Column 8 records $T^c_{H_2O}$ (corrected to 1.73 sq. m. S.A.) as determined arbitrarily at a urine flow of 20 ml. per min. Columns 9, 10, and 11 record the extremes of plasma osmolarity, sodium concentration and potassium concentration, respectively, at the minimal and maximal urine flows used in the calculations of columns 6 and 7. The average pre-infusion values of these terms are given at the bottom of the table. The last column records the average inulin clearance (corrected to 1.73 sq. m. S.A.) during the entire duration of the mannitol infusion.[9]

The most striking feature of these data is the high coefficient of correlation for linear regression, which is invariably in excess of 0.995 (see column 6 of Table I), implying both considerable reliability in the individual observations and regularity in the underlying physiological processes.

The next most striking feature is the extent to which the slope of the regression lines in various subjects approaches unity; the mean slope in the 21 subjects in Table I is 1.033 ± 0.075, yielding a coefficient of variation of only 7 per cent. In 15 out of 21 subjects, the deviation of slope from 1.00 is less than $\pm$ 10 per cent. The extent to which the slope approaches 1.00 over a wide range of urine flow is substantial evidence that the concentrating process operates basically by the extraction of an approximately constant quantity of water (identified in Figure 1 as $T^c_{H_2O}$) from a variable volume of isosmotic urine. However, since the slope of the regression line in some subjects does in fact deviate significantly and consistently from 1.00, $T^c_{H_2O}$ must tentatively be determined at an arbitrarily selected urine flow. For the purposes of this discussion only, we have chosen a urine flow of 20 ml. per min. At this urine flow, $T^c_{H_2O}$ in these 21 subjects has an average value of 5.1 ml. per min. per 1.73 sq. m. body surface area. This average value has a high standard deviation (1.5) and coefficient of variation (29.4 per cent), but it must be emphasized that nothing is known concerning the possible effects of age, sex, diet, nutrition, or other factors on the concentrating operation, and also that the calculation at a urine flow of 20 ml. per min. is itself arbitrary.

Between the lowest and highest urine flow, the average plasma *osmolarity* increased (from 288 to 295 mosm per liter, as shown in column 9) in consequence of the infusion of hypertonic mannitol. This represents an increase of about 2.4 per cent and, added to pre-existing hydropenia, may

[7] Presumably this operation is carried out low in the nephron, after the glomerular filtrate is substantially reduced in volume by isosmotic salt and water reabsorption.

[8] As calculated from 10 subjects in Group I, mannitol supplies about two thirds of the osmotic pressure of the urine at a urine flow of 20 ml. per min.

[9] Until near the conclusion of this work, commercial inulin was not routinely tested for hydrolysis, and it was subsequently discovered that in some of the ampouled material then in use a considerable fraction of the 'inulin' was fermentable by yeast and represents low molecular weight oligosaccharides, a circumstance that may have given erroneously low figures for the filtration rate. We therefore place little emphasis on this datum except as reflecting changes in filtration rate in any one subject.

have served to sustain endogenous antidiuretic hormone secretion.

The average plasma *sodium concentration* decreased from 135 to 127 mEq. per liter in consequence of dilution of body fluid by the infusion, withdrawal of water from the cells, and increased urinary excretion of sodium. No clinical evidences of hyponatremia were encountered.

The average plasma *potassium concentration* increased from 4.50 to 4.90 mEq. per liter.

The *filtration rate* underwent negligible changes in consequence of the mannitol infusion, some subjects showing a slight decrease toward the end of the experiment, others showing a slight increase.

Pitressin in variously hydrated subjects

In 13 of the 21 subjects reported above (7 male, 6 female) a second series of observations was made with mannitol plus Pitressin immediately after completion of the observations with 10 per cent mannitol alone. At the maximal urine flow shown in Table I, the mannitol infusion was stopped and the subject was given one liter of water to drink to ameliorate the rather severe dehydration occasioned by the preceding osmotic diuresis. Pitressin (Parke, Davis & Co.) was given intravenously in a priming dose of 100 mU over a period of 3 to 4 minutes, followed by a sustaining infusion at a rate of 1 to 2 mU per Kg. per hr. up to the end of the test.[10] One to one and one-half hours after the ingestion of water, at a time when the urine flow had fallen to some 8 to 10 ml. per min. in consequence of the elimination of the mannitol that had been administered previously, the second infusion of mannitol was started. Seven of the 13 subjects received 10 per cent mannitol at a rate of 20 ml. per min. (Subjects Nos. 1–6 and 15); the rest received a 5 per cent solution at rates of 30 ml. to 40 ml. per min., the intention being to attain as high a urine flow as possible within the practicable time of 1 to 1½ hours. The observations on these 13 subjects are recorded in Table II.

[10] Possible inactivation of Pitressin in the infusion was examined in two subjects by doubling the sustaining dose in one (No. 24 see Figure 1) and by introducing a fresh solution midway in a test in the other one. There was no change in slope or $T^c_{H_2O}$.

TABLE II

Re-examination of 13 of the subjects reported in Table I. Osmotic diuresis (5 and 10 per cent mannitol) in variously hydrated subjects with the addition of Pitressin

1 Subject	2 Sex	3 Age	4 S.A.	5 Urine flow range	6 Correl. coeff.	7 Regres. coeff. (slope)	8 $T^c_{H_2O}$ per 1.73 sq. m.	9 P_{osm} range	10 P_{Na} range	11 P_K range
		yrs.	*sq. m.*	*ml./min.*			*ml./min.*	*mosm/L.*	*mEq./L.*	*mEq./L.*
1*	M	22	1.70	9.2–16.4	.9998	1.153	8.5	283–286	141–133	4.90–4.4
2*	M	15	1.60	9.9–24.9	.9949	1.034	6.7	282–283	129–127	4.6 –4.6
3*	M	18	1.73	9.1–21.9	.9970	1.006	6.6	287–288	–125	–4.7
4*	F	43	1.72	10.2–20.0	.9992	.999	5.7	296–301	123–120	4.9 –4.6
5*	F	18	1.58	13.5–32.8	.9997	.948	7.8	294–298	130–120	5.0 –4.8
6*	F	34	1.46	11.7–24.2	.9998	.999	5.9	305–303	128–121	4.7 –4.0
10†	M	21	1.49	10.0–21.2	.9982	.928	6.2	277–280	128–122	4.5 –4.3
11†	M	46	1.65	10.5–19.4	.9984	.940	5.3	292–293	125–118	–4.5
12†	M	27	1.85	11.2–24.8	.9931	.868	5.5	289–281	128–121	4.2 –4.4
13†	F	30	1.57	11.8–24.5	.9996	.934	3.1	283–283	125–121	4.5 –4.6
14†	F	24	1.78	16.8–42.5	.9997	.832	2.1	292–289	129–121	4.8 –4.7
15†	F‡	27	1.62	9.7–23.3	.9997	1.007	8.0	281–293	136–118	4.0 –4.2
16†	M	18	1.56	12.0–18.3	.9962	1.036	2.8	287–296	133–117	4.5 –4.5
Mean value				11.2–24.2	.9973	.976	5.7	288–290	129–122	4.6 –4.5
Standard deviation						± .084	±2.0			
Coefficient of variation						0.086	0.35			

Corresponding mean values, standard deviation and coefficient of variation for the same 13 patients during hydropenia alone, calculated for these subjects as reported in Table I:

				9.5–23.3	.9992	1.020	4.9	288–303	138–129	4.6 –4.8
						±0.070	±1.8			
						0.069	0.36			

* 10 per cent mannitol solution.
† 5 per cent mannitol solution.
‡ Not rehydrated.

TABLE III

Osmotic diuresis in hydrated subjects receiving Pitressin and 5 to 12.5 per cent mannitol solution

1 Subject No.	2 Sex	3 Age	4 S.A.	5 Urine flow range	6 Correl. coeff.	7 Regres. coeff. (slope)	8 $T^c_{H_2O}$ per 1.73 sq. m.	9 P_{osm} range	10 P_{Na} range	11 P_K range
		yrs.	*sq. m.*	*ml./min.*			*ml./min.*	*mosm/L.*	*mEq./L.*	*mEq./L.*
8	F	30	1.42	7.4–15.7	.9996	.966	5.0	276–272	125–	
9	M	31	1.79	7.4–12.2	.9941	.991	4.1	276–273	121–111	4.5–4.7
17	F	29	1.76	12.2–34.9	.9998	.874	3.7	275–275	130–121	4.2–4.0
28	M	25	1.83	8.3–21.3	.9986	.990	4.8	279–281	124–103	4.2–4.2
29	F	30	1.60	8.5–31.0	.9993	.863	2.2	277–284	134–121	4.8–
30	F	36	2.05	3.6–23.6	.9991	.855	3.8	285–290	144–134	4.1–4.0
31	F	25	1.65	6.5–23.8	.9990	.924	5.2	274–284	134–119	4.6
32	F	35	1.46	9.9–25.9	.9998	.874	3.6	279–281	132–120	4.3
Mean value				8.0–23.6	.9987	.917	4.1	278–280	131–116	4.3–4.2
S.D.						±.058	±1.0			
Coefficient of variation						0.064	0.24			
Two hydropenic subjects receiving Pitressin and hypertonic (10 per cent) mannitol:										
24	F	43	1.58	5.9–41.4	.9997	1.063	7.6	291–315	132–119	4.1–4.3
25	F	27	1.62	11.4–31.4	.9995	1.000	5.1	293–312	126–121	4.6–5.1

As between hydropenia alone and hydropenia combined with Pitressin, the mean slope changed from 1.033 to 1.020, a change to which no significance can be attached at this time. The slope decreased under Pitressin in 11 subjects, and increased in 2.

The mean value of $T^c_{H_2O}$ increased from 4.9 ± 1.8 during hydropenia to 5.7 ± 2.0 during Pitressin. Nine subjects showed an increase in this value, 2 showed a decrease, and 2 did not change. Whether the change in mean value is significant is difficult to say because of the small number of subjects studied. Moreover, the conditions of the two tests with respect to the plasma sodium concentration and final osmolarity were not identical; also in the second series the subjects were initially in a more dehydrated state than in the first, despite the administration of water, a circumstance that may have influenced the concentrating mechanism itself. Therefore, we do not believe that the differences in the two series with respect to $T^c_{H_2O}$ necessarily indicates that Pitressin plus hypertonic mannitol induces more effective antidiuresis than hydropenia alone, an inference which is borne out by more critical experiments to be reported later.

Pitressin superimposed on hydration

Eight subjects (2 male, 6 female) in various degrees of hydration (breakfast with coffee or in some instances as much as a liter of water) were examined with 5 to 12.5 per cent mannitol at infusion rates ranging from 20 to 40 ml. per min. and Pitressin in a priming dose of 50 to 100 mU followed by a sustaining infusion (Table III). This group shows the lowest average slope (0.917 ± 0.058) of any in our series. The mean value of $T^c_{H_2O}$ (4.1 ± 1.0) in this series is lower than that recorded in Table I (5.1 ± 1.5). The group, however, is small and contains a preponderance of females (in whom all reported renal functions are less than in males), and again we cannot argue confidently that this difference has physiological significance.

Also recorded in Table III are observations on two hydropenic women (Nos. 24 and 25) infused with 10 per cent mannitol and Pitressin as described in the preceding paragraph. Since they received Pitressin they cannot be included with the patients listed in Table I, and since they were hydropenic they cannot be included with the subjects listed in Tables II and III. Both show a slope and correlation coefficient close to 1.0. Subject 24 is recorded in Figure 1.

Reproducibility in two consecutive tests made under identical conditions and on the same morning was examined in one hydropenic subject employing hypertonic mannitol alone (No. 7), and in two hydrated subjects employing isotonic mannitol and Pitressin in the priming and sustaining dosage used before. $T^c_{H_2O}$ per 1.73 sq. m. increased from 4.2 in the first test to 5.3 in the sec-

ond test in the hydropenic subject, possibly because of the cellular dehydration occasioned by the first test. In the two hydrated subjects $T^{c}_{H_2O}$ showed no change between the two tests.

Pitressin given in the course of hypertonic mannitol infusion

In order to eliminate complicating factors such as variable degrees of dehydration, etc., which are difficult to avoid in duplicate tests on the same subjects, Pitressin was added to an infusion of hypertonic mannitol midway in a continuous test in 6 hydropenic subjects. The first part of the test for these 6 subjects is reported in Table I (Nos. 18 to 23). Pitressin (priming and sustaining as described before) was added at the time when the maximal urine flow, as recorded in Table I, had been attained, and without changing the rate or concentration of the mannitol infusion (10 per cent). Observations were continued for 3 to 10 urine collection periods, at which time the urine flow had reached rates of 25 to 30 ml. per min.

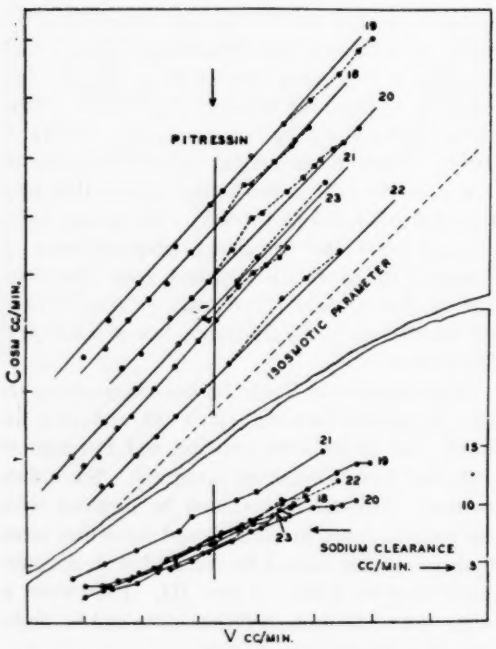

FIG. 2. THE EFFECTS OF PITRESSIN ON SLOPE AND $T^{c}_{H_2O}$ WHEN ADDED IN THE COURSE OF A HYPERTONIC MANNITOL INFUSION IN HYDROPENIC SUBJECTS

The data on osmolar clearance and urine flow are plotted electively in order to bring into coincidence the time of Pitressin administration, and consequently the actual values of $T^{c}_{H_2O}$ are not revealed by the graph. The slope, however, is not affected by this method of plotting. The data on the sodium clearance represent absolute values.

The results of these observations are illustrated in Figure 2, where the data on osmolar clearance and urine flow are plotted in such a manner as to bring into coincidence the time of Pitressin administration. These data show that the addition of Pitressin to a hypertonic mannitol infusion in hydropenic subjects has at most a slight effect on either the slope or $T^{c}_{H_2O}$ (the actual values of the latter are not revealed in Figure 2 because of the use of elective coordinates). The observed deviations are fleeting in nature and small in magnitude, and appear to be referable to secondary, unidentified factors. We interpret these observations as indicating that hydropenia plus hypertonic mannitol produces maximal antidiuresis, and that the larger series of observations reported in Table I were obtained under maximal antidiuresis. This statement probably also applies to the subjects reported in Tables II and III with the qualification that the physiological status of these subjects was not the same as in those reported in Table I.

As a matter of interest, we have included in Figure 2 the sodium clearance, as related to urine flow, also plotted electively so as to bring into coincidence the administration of Pitressin. It is known that osmotic diuresis increases sodium excretion, a fact shown here by the progressive increase in sodium clearance as the urine flow increases. The administration of Pitressin does not appear to modify the uniformity of this relation.

It may also be recorded that Pitressin did not change the filtration rate, as shown by comparing the averages of two 10 min. periods immediately before and after Pitressin. The differences ranged from -1 to $+8$ ml. per min., which fall within the error of the method. In this observation we confirm Maxwell, Breed, and Smith (9).

The results presented in Figure 2 show that as between hydropenia plus hypertonic mannitol, on the one hand, and hydropenia plus mannitol and Pitressin on the other, there are no essential differences in slope, $T^{e}_{H_2O}$, or sodium excretion, nor

is there a perceptible change in the filtration rate, and it is therefore inferred that the action of Pitressin (in physiological doses) on the kidney is identical with that of the endogenous antidiuretic hormone. We believe that all our data are in agreement with this conclusion.

Effect of Mercuhydrin®

When 1 ml. of Mercuhydrin® was administered intravenously to 2 hydropenic subjects midway in time during 10 per cent mannitol plus Pitressin infusion, urine flows of 31 and 35 ml. per min. were reached, with no change in $T^c_{H_2O}$ in one patient and possibly a slight decrease in the other. This observation confirms Ladd's (5) conclusion that Thiomerin® has no effect on the reabsorption of such water as is involved in the renal concentrating operation, a conclusion which may be inferred from the data of Welt, Goodyer, Darragh, Abele, and Meroney (10) though it is not so stated by these writers. Similarly, Page, Scott-Baker, Zak, Becker and Baxter (14) have found that Thiomerin® is without effect on the concentrating mechanism in the marine seal, *P. vitulina*.

DISCUSSION

Slope of the regression line relating C_{osm} to V

For $T^c_{H_2O}$ to be constant, it is required that the slope of the curve relating C_{osm} to V must be 1.00 at all urine flows. The mean slope in the 31 subjects (derived from single tests or the first of duplicate tests) is 1.003 ± 0.083 (coefficient of variation 8.5 per cent), but the range is from 0.83 to 1.16.

If one allows an error of 5 per cent for methodological variations, $T^c_{H_2O}$ has been found to be constant in only 12 out of 31 subjects (counting only single tests or the first one of duplicate tests on the same subject on the same day) and in only 23 out of 44 tests counting all tests separately. With respect to slope, the 31 subjects referred to above are nearly equally divided above and below 1.0.

In considering the possible reasons why the slope deviates from 1.0 in some subjects, it is important to note that any one subject, during consecutive observations extending over a period of two to three hours, reveals remarkable constancy in behavior as shown by the fact that the coefficient of correlation (relative to linear regression) of C_{osm} on V is invariably above 0.995, and frequently above 0.999.[11] This constancy in behavior is apparent even in subjects with a slope significantly greater or less than 1.0, as is shown by No. 24 in Figure 1, where the slope is 1.063 and the correlation coefficient is 0.9997, and by No. 14, who on two occasions showed the lowest slope of any subject studied (0.863 in Table I and 0.832 in Table II), and for whom the correlation coefficient was 0.9998 and 0.9997, respectively. This subject is illustrated in Figure 3, and it will be seen that in one test slightly hypotonic urine was excreted at urine flows above 33 ml. per min. This consistency suggests that we are dealing with factors which systematically affect the slope and which continue to operate in a regular manner in any one subject in observations extending over a period of several hours, and despite marked changes in urine flow.

Several factors which might contribute to variability in slope have been considered, but no one of them, or no two of them in combination, can be reconciled with the experimental data. A slope less than 1.0 could be explained during antidiuresis by the delivery of hypotonic urine to a distal concentrating mechanism which operated under the limitations of a fixed rate of reabsorption of water; but this explanation would require that the tubular urine/plasma osmotic ratio remain constant despite wide changes in urine flow induced by osmotic diuresis, a condition which renders the explanation implausible. Loss of Pitressin activity or too small a dose of Pitressin has been excluded as a cause of a slope less than 1.0 (Nos. 17 and 24).

[11] A similar high coefficient of correlation is shown between $U_{Na}V/kP_{Na}$ wC_F and V/wC_F in Ladd's (5) study of saline diuresis in prehydrated subjects who were receiving Pitressin.

In Ladd's studies, $T^c_{H_2O}$ as calculated from sodium excretion alone had a value of only 2 ml. per min. per 100 ml. of glomerular filtrate. We cannot make an exact comparison with our figures because of some uncertainty in our data for the inulin clearance, but our average figure is 5.1 ml. per min. per 100 ml. of filtrate, a figure that is probably not very much in error. The remarkable difference in these figures may possibly be related to the fact that Ladd's subjects were prehydrated 8 to 13 hours before or to the fact that the loading substance was NaCl in Ladd's study.

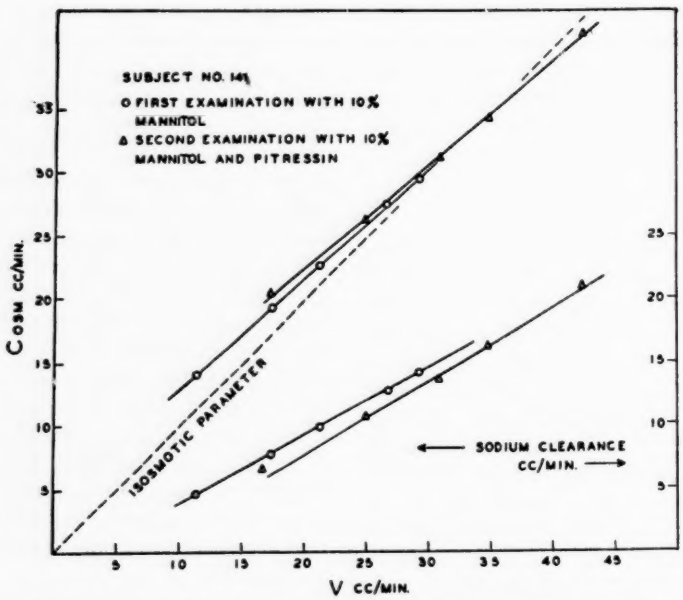

FIG. 3. DUPLICATE EXAMINATION OF ONE SUBJECT, FIRST IN THE HYDROPENIC STATE WITHOUT AND THEN WITH PITRESSIN AFTER PARTIAL REHYDRATION

Hypertonic mannitol was used in the first test, isotonic in the second. This subject showed the lowest slope of all subjects examined.

Conversely a slope greater than 1.0 could be explained by the delivery to the concentrating mechanism of urine which is consistently hypertonic, but this speculation is contrary to such information as is available on the composition of the urine in the proximal tubule.

A consistent over- or underestimation of the plasma osmolarity (method error) can be ruled out as causing the slopes to differ from 1.0, as both the plasma and the urine samples of each patient were measured consecutively and no longer than 2 to 3 hours after conclusion of the experiment.

Gradual attainment of the maximal antidiuretic state would increase the slope above 1.0, but our observations indicate that maximal antidiuresis is attained early in the tests and is not significantly enhanced by the administration of Pitressin. A slope greater than 1.0 would also be obtained if maximal activity in the concentrating mechanism were approached asymptotically with increasing urine flow, but the fact that the correlation coefficient is greater than 0.999, even in those subjects in whom the slope of the regression line exceeds 1.0, argues against this assumption.

Dead space error of significant magnitude would tend to lower the slope during ascending urine flows, but in a non-linear manner and with negligible effects at high urine flows. In no case could it produce a slope above 1.0.[12] Nor does it seem possible to explain the deviations in slope by progressive changes in the volume of the dead space, or by the use of an incorrect delay time correction.

As matters stand, therefore, we are unable to find any satisfactory explanation for the fact that the slope of the regression line of C_{osm} on V shows the range it does.

Throughout the experimental part of this paper

[12] All observations reported in the body of the paper were made during increasing osmotic diuresis, but two subjects were also examined during descending diuresis. In both individually, the slope was slightly less during the descending than during the ascending phase, a result contrary to what would be anticipated if dead space error were significant.

we have used the designation $T^c_{H_2O}$ to indicate the water abstracted from the urine in raising it to the hyperosmotic state. We believe that our observations can be interpreted as indicating that during antidiuresis the concentrating mechanism operates essentially by the removal of a constant quantity (in ml. per min.) of solute-free water. The magnitude of this constant quantity we consider a fixed renal function under carefully controlled circumstances, a qualification which it is difficult to realize experimentally. This hypothesized function can be designated as $T^c_{mH_2O}$, the addition of the letter *m* indicating that the operation represented by $T^c_{H_2O}$ has a limiting maximal value. This limiting maximal rate, however, may be subject to secondary factors and if such arise in the course of the experiment (*e.g.*, progressive dehydration) the slope of the regression line will be affected.

Limiting osmotic U/P ratio

The relationship indicated by the regression line of C_{osm} on V obviously cannot hold when the urine flow has a value less than $T^c_{H_2O}$ and at low urine flows a new limitation, the maximal osmotic U/P ratio, is imposed on the process of urinary concentration (8).[13] A constant osmotic U/P ratio requires direct proportionality between C_{osm} and V, and data conforming with a constant osmotic U/P ratio will fall on a regression line intersecting the ordinates and abscissae at zero, such as the short line shown at the extreme left of Figure 1. No special effort has been made in this study to examine the maximal osmotic U/P ratio at low urine flows, but it is apparent from unpublished experiments that a maximal osmotic U/P ratio can be reached only when the osmolar clearance is small, and that the transition from the one regression line to the other is gradual and characterized by significant splay. It is because of this gradual transition from one regression line to the other that we have necessarily excluded from our analysis data at lower urine flows where this transitional area has been entered. The lower limit of V at which linear regression ceases has ranged from 3 to 8 ml. per min. in different individuals.

Osmotic U/P ratio during osmotic diuresis

As the urine flow increases during antidiuresis in consequence of an increasing osmolar clearance, the osmotic relations between blood and urine pass gradually from the parameter determined by the limiting osmotic U/P ratio to the parameter determined by $T^c_{H_2O}$, so that the osmotic U/P ratio decreases asymptotically from the maximal osmotic U/P ratio to approach a value of 1.0 at large urine flows, giving rise to the approximately hyperbolic relationship originally described by Hervey, McCance, and Tayler (11) and subsequently by Rapoport, Brodsky, West, and Mackler (12).

Observation on the dog and seal

Since this work was initiated, a similar investigation in the dog has been completed and reported from this laboratory by Page and Reem (13), who conclude that at urine flows ranging from 11 to 28 per cent of the filtration rate, $T^c_{H_2O}$ had reached maximal values (7.6 and 6.2 ml. per min. per 100 ml. of filtrate), as judged by constancy and reproducibility, but the data do not rule out an asymptotic approach to these or slightly higher values. In the seal, Page, Scott-Baker, Zak, Becker, and Baxter (14), utilizing hydropenia, saline diuresis, and Pitressin, have found that at the highest values of C_{osm} attainable (16 to 18 per cent of the filtered load), an apparent maximal value of $T^c_{H_2O}$ was attained in only one animal—in all others this value continued to increase with increasing urine flow. This is perhaps related to the special adaptations of the seal as a mammal which lives on metabolic water, and to the fact that the maximal value of $T^c_{H_2O}$ must be as much as 10 ml. per min. per 100 Gm. kidney weight as

[13] In the majority of our subjects the osmotic U/P ratio in the control urine after 12 to 14 hours without fluids ranged from 2.5 to 3.87. Most of our figures are significantly lower than those reported by other investigators, for reasons unknown to us.

A possible explanation may be the following: All the patients were ambulatory. On reaching the 'unit' after a 3-minute walk they were put to bed and catheterized. The urine found in the bladder was discarded and the control urine was collected over a period of 20 to 30 minutes. We have noticed in several patients that the control urine elaborated in the supine position was markedly lighter in color than the discarded urine elaborated during the erect position; this was particularly striking in 2 rather anxious patients.

compared to 3.8 to 5.4 ml. in the dog, and some 1 to 2 ml. in man. It is possible that failure to attain maximal values of $T^c_{H_2O}$ in the seal is related to the relatively large magnitude of this function.

SUMMARY

1. The urinary concentrating process has been examined in 54 tests on 31 normal subjects in the antidiuretic state by the induction of osmotic (mannitol) diuresis at urine flows ranging from 5 to 40 ml. per min. Antidiuresis was induced either by abstinence from fluids for 12 to 14 hours (hydropenia), by hydropenia supplemented by Pitressin, or by the administration of Pitressin to hydrated subjects.

2. The degree of osmotic concentration of the urine was determined by application of the cryoscopic method to plasma and urine, and the calculation of the quantity of water (in ml. per min. and here designated as $T^c_{H_2O}$) required to restore the urine to an isosmotic state with respect to the glomerular filtrate.

3. The conclusion is reached that the concentrating mechanism operates essentially by the removal of a constant, maximal quantity of solute-free water irrespective of the urine flow, so long as the latter somewhat exceeds this maximal reabsorptive rate.

4. The presumed constancy of $T^c_{H_2O}$ requires that the regression line generated by plotting the osmolar clearance (C_{osm}) against the urine flow (V) should have a slope of 1.00. In 31 subjects during a first examination on any one day, the slope averages 1.003 ± 0.083. In 12 of these subjects it has a value between 0.95 and 1.05, which we do not consider to be significantly different from 1.0. In the remaining 19 subjects the slope deviates from 1.0 by more than $\pm$ 5 per cent, with extremes of 0.83 and 1.16. It is believed that unknown factors operate systematically in some individuals or under certain circumstances to cause slight, regular increases or decreases in $T^c_{H_2O}$ with increasing diuresis.

5. As opposed to this apparent inconstancy of $T^c_{H_2O}$ under our experimental conditions, the consistency of behavior of any one individual is remarkable, as shown by the fact that in all subjects, despite protracted observations and rather unphysiological circumstances, the coefficient of correlation (relative to linear regression) of C_{osm} on V is invariably above 0.995, and frequently above 0.999.

6. This concentrating operation appears to be generally identical whether the antidiuretic state is induced by endogenous antidiuretic hormone, by Pitressin in physiological doses, or both.

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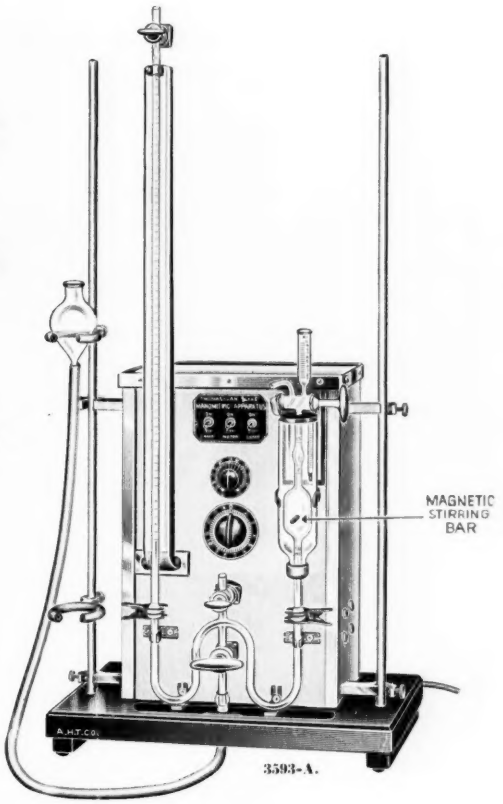

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RENAL FAILURE DUE TO CALCIUM AND ABSORBABLE ALKALIES FOR PEPTIC ULCER

Clinical Investigation

More than thirty years ago, Hardt and Rivers[1] pointed out that the excessive ingestion of calcium and absorbable alkalies for the relief of peptic ulcer may induce systemic alkalosis and chronic renal failure. These observations have been repeatedly confirmed.[2, 3, 4] More recently, in addition to the alkalosis and kidney dysfunction, metastatic calcification has been reported.[5, 6, 7] Dufault and Tobias[8] regard the typical syndrome as potentially embracing this triad of manifestations.

PATHOGENESIS

In the opinion of Dufault and Tobias, the prerequisite for the development of metastatic calcification is either hypercalcemia or alkalosis or both. In their series, alkalosis was associated with hypercalcemia in three cases out of four. Although Wermer[6] reports that the role of alkali intake appears uncertain, he found a history of concomitant ingestion of excessive calcium and alkali in all cases reviewed in the literature. Wermer believes that the increased urinary excretion of calcium in hypercalcemia may induce tubular calcification and consequent renal impairment, with retention of phosphate in the serum. The elevated blood levels of these calcium and phosphate ions may then induce metastatic calcification in extrarenal tissues.

HISTORY

All four patients observed by Dufault and Tobias had had peptic ulcer for more than twenty years. The condition had been managed by a daily intake of from 1 to 4 quarts of milk and large quantities of absorbable alkalies (sodium bicarbonate, calcium carbonate). Intensive therapy for at least eight years on this regimen was followed by symptoms secondary to hypercalcemia and renal failure.

DIAGNOSIS

Laboratory examination showed impairment of kidney function with azotemia, normochromic and normocytic anemia, hypercalcemia, normal or elevated phosphorus, normal alkaline phosphatase and potassium with low chloride levels. In three cases, the total bicarbonate level of the blood was above normal. Ocular calcification—the most reliable physical sign of the syndrome, according to the authors—was found in three patients.

TREATMENT

After institution of a low-calcium diet and the use of nonabsorbable antacids, symptomatic improvement occurred in all patients. In two, renal function returned to normal. In the other two patients, however, the kidney damage had become irreversible, and one of them died. Since recovery depends on the extent of renal impairment, the authors stress early diagnosis and treatment, as well as prevention, "which precludes the prolonged use of absorbable alkalies and excessive calcium."[8]

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